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ASSESSING CONTAMINANT SENSITIVITY OF AMERICAN SHAD, ATLANTIC STURGEON AND SHORTNOSE STURGEON

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by

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Action Plan Project

Hudson River Estuary

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NOTICE

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EXECUTIVE SUMMARY

The Hudson River Estuary supports the largest stocks of Atlantic sturgeon (*Acipenser oxyrhynchus*) and American shad (*Alosa sapidissima*) on the Atlantic coast. Current stocks for both species are reduced from historic populations. Recent declines have been attributed to over-fishing. Issues concerning the effects of over-fishing have been addressed in interstate management forums for both species. The coast-wide Atlantic sturgeon fishery was closed in 1997 and issues regarding shad are ongoing. Concern still remains for both stocks including effects on habitat conditions that could potentially affect the spawning stock and future production. Concerned scientists and citizens provided impetus for this study which assesses issues related to environmental contaminants and their possible effects on stock recovery.

In 1997 USGS, in cooperation with NYSDEC, began a series of studies to evaluate the sensitivity of American shad and Atlantic sturgeon to contaminant exposures. In the first assessment, acute toxicity tests (96-h LC50) were conducted with early life-stages of American shad, Atlantic sturgeon, and shortnose sturgeon (*Acipenser brevirostrum*) using different classes of chemicals and modes of toxic action. These chemicals have been tested in previous cooperative research conducted between the U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, and U.S. Geological Survey for the same five chemicals with early life-stages of rainbow trout, fathead minnows and 13 different threatened and endangered species. After 48 h of exposure to carbaryl, copper, pentachlorophenol and permethrin, the LC50s for the American shad were lower than the 48-h LC50s for the standard test organisms, rainbow trout and fathead minnow. However, the difference between American shad and rainbow trout may not be significant. The results for the American shad should be interpreted with caution given the high control mortality after 48 h of exposure. Results for tests conducted with the two species of sturgeon indicate that the sturgeon are somewhat more sensitive to contaminant exposure than are the rainbow trout. However, sturgeon were also difficult to test, and conclusions regarding the chemical sensitivity of the sturgeon also need to be interpreted with caution.

For the second assessment, 96-h water-renewal toxicity tests were conducted using standard effluent test procedures. Attempts were made to conduct 96-h survival studies with embryo-larval fathead minnows and analogous exposures using American shad and Atlantic sturgeon. Effects on survival of the cladoceran *Ceriodaphnia dubia* were also evaluated. Tests were conducted with two effluents collected from discharges into the Hudson River, New York. From the results obtained in this study, the fathead minnow survival test appears to be a reliable estimator of effects to American shad and Atlantic sturgeon. However, neither the American shad nor the Atlantic sturgeon were suited for the procedure used in this study. Unacceptable mortalities occurred among controls. Future use of these species for testing require development of alternate culture and testing techniques. Factors such as handling procedures, optimum feeding rates, optimum test temperature, expected test to test variation and expected survival would need to be established.

Given the difficulty in testing the Atlantic sturgeon and American shad, results from this study should be interpreted very cautiously. However, if used as a preliminary estimate of sensitivity to contaminant exposure, then the results from this study would indicate that these two species are sensitive to exposure to environmental contaminants. Results from this study should be verified by additional method development and testing.

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BACKGROUND

The Hudson River estuary supports the largest stocks of Atlantic sturgeon (*Acipenser oxyrhynchus*) and American shad (*Alosa sapidissima*) on the Atlantic coast. Concern for the status of Atlantic sturgeon stocks began in earnest in 1995 with the passage of the Atlantic Coastal Cooperative Fisheries Management Act. Only a few viable, self-supporting stocks of Atlantic sturgeon exist, the Hudson's being the largest. Production of juvenile Atlantic sturgeon in the Hudson estuary declined dramatically in the 1980s as fisheries in both coastal waters and in the river grew. An assessment of stock status determined over-fishing was the primary cause for the decline and which resulted in a coast-wide moratorium on fishing in 1997.

Over the same time period, similar concerns for the American shad stock grew as in-river landings declined in the late 1980s. By 1991, the Hudson shad spawning stock population indicated typical signs of over-fishing, a lack of older fish in the population from what had been prevalent just a few years earlier (Hattala and Kahnle 1998). The causes for decline in the Hudson shad continues to be debated in the interstate management forum.

Concerned citizens, primarily fishers whose livelihood was threatened, along with other individuals and scientists suggested that habitat change or exposure to environmental contaminants caused or contributed to the stock declines of both species (Hudson River Estuary Advisory Committee, personal communication). Their concerns provided the basis to initiate this study.

Study area

The Hudson River estuary (Figure 1) extends north about 246 km from the Battery at New York City (km 0), to the Federal Dam at Troy (km 246).

The river, by nature, is slow moving with freshwater flows being much smaller than tidal flows (Dramer 1969). The

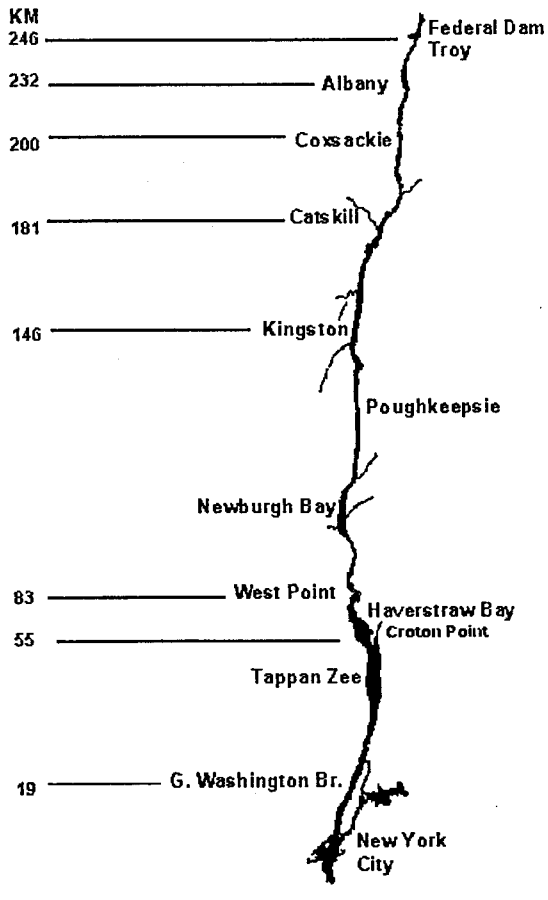

Figure 2. Hudson River estuary.

tides affect the entire length of the estuary below the Federal Dam. Mean tidal ranges vary from 0.8 m near West Point to 1.4 m at Albany (U.S. Dept. of Commerce 1995). Current velocities during ebb and flood tides range between 0.5 and 0.9 m/s, depending on tidal stage and section of river (U. S. Dept. of Commerce 1995).

Maximum widths of the estuary range from 5.5 km wide in Haverstraw Bay to 2.5 km in Newburgh Bay. The estuary, north of Newburgh Bay, narrows to less than 1.6 km and is characterized by numerous shoals and sand bars throughout. A shipping channel is maintained at 9.8 m allowing passage of ocean-going commercial vessels to the Port of Albany (km 232). The deepest section of the river occurs near West Point (km 83), where depth averages 48.8 m. The location of the salt front varies seasonally with freshwater inflow. In late summer, the salt front usually extends as far north as Newburgh Bay (km 90), in drought years it can located as far north as Poughkeepsie (km 125).

The section of River from Poughkeepsie (km 125) north to Catskill (km 181) is used as a drinking water source for many Hudson Valley communities. This area falls within a river reach classified by the NYSDEC as "A" waters (NYCRR 6, Part 858), suitable for drinking or food processing. The class "A" waters of the Hudson extend from upper Newburgh Bay north to Houghtaling Island, north of Coxsackie, NY. Sewage treatment plants in this river reach are required to disinfect their discharge to meet this classification. Chlorine is generally the disinfectant of choice; however, upgrades of many plants may include the use of ultraviolet light. North and south of this river reach, discharges are not disinfected. North of the "A" waters to the confluence of the Mohawk River with the Hudson above Troy, the classification is "C"; best use is fishing. Below Newburgh, waters are class "B"; best use is primary and secondary contact recreation and fishing. Newburgh Bay, north to Troy encompasses the spawning areas of the species examined in this study.

Life history of American shad and Atlantic sturgeon

Adult American shad typically enter the Hudson River estuary in late March or early April (McFadden 1977). Peak migration into the estuary occurs from mid-April through mid-May at water temperatures of 7 to 14°C (Talbot 1954). Spawning occurs at temperatures from 14 to 20°C from Port Ewen (km 145) to Coxsackie (km 200), but is concentrated near Catskill (km 181); (Talbot 1954). Following spawning, most adults leave the estuary (McFadden 1977). Larvae and young-of-the-year shad disperse to nursery areas from Newburgh (km 90) to Albany (km 232) in early summer (Hattala et al. 1988). Young-of-year shad leave the Atlantic coastal rivers when water temperatures drop below 15°C for several days (Leggett and Whitney 1972).

Adult Atlantic sturgeon begin their spawning migration into the Hudson River estuary in the month of May and continue until August (Dovel and Berggren 1983). Spawning occurs at temperatures from 14 to 20°C from Newburgh Bay (km 90) to Catskill (km 181). Concentration areas are not well known, but occur within this river reach. Following spawning, most adults leave the estuary, but some remain within the estuary from late summer through the early fall. Nursery areas for larvae and young-of-the-year shad are not well known, but are assumed to be within the spawning reach. Juvenile Atlantic sturgeon remain in the Hudson River estuary for

several years and slowly emigrate to ocean waters of the Atlantic coast from ages three to five (Dovel and Berggren 1983).

Contaminant sensitivity

In order to evaluate the impact of a contaminant release into the environment, standardized toxicity tests are conducted using standard organisms as surrogates for other species (USEPA 1982). Inherent in these tests is the assumption that the test species used for toxicity assessments are predictive of other species. Surrogate species are typically organisms that are easily tested using standardized methods. However, these species may not be representative of all species. The wide use of pesticides and other commercial chemicals invariably poses a risk to aquatic species in decline since, by definition, their distribution is limited and further adverse effects on these populations could lead to extinction. Species may be under protected, or unnecessary regulatory programs may be implemented, if the sensitivity of these species is not evaluated.

Objectives

The following research project provides information for assessing contaminant sensitivity of American shad and Atlantic sturgeon. The sensitivity of shortnose sturgeon (*Acipenser brevirostrum*) was also evaluated. Two different assessments were conducted.

Objective 1: Evaluation of the sensitivity of American shad, Atlantic sturgeon and shortnose sturgeon to different classes of toxicants.

Objective 2: Evaluation of the use of standard effluent toxicity tests for protection of American shad and Atlantic sturgeon.

Information on the sensitivity of these species to contaminant exposure can be used to help establish appropriate regulatory procedures for their protection.

Chapter 1: Evaluation of the sensitivity of American shad, Atlantic sturgeon and shortnose sturgeon to different classes of toxicants.

INTRODUCTION

Acute toxicity tests (96-h LC50) were conducted with early life-stage American shad, Atlantic sturgeon, and shortnose sturgeon using five chemicals having different toxicological modes of action. Chemicals included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. These chemicals have been tested in previous cooperative research conducted between the U.S. Environmental Protection Agency (USEPA 1995), U.S. Fish and Wildlife Service (USFWS), and U.S. Geological Survey (USGS) for the same five chemicals with early life-stage rainbow trout, fathead minnows and 13 different threatened and endangered species - Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, razorback sucker, fountain darter, greenthroat darter, shovelnose sturgeon, gila topminnow, boreal toad, spotfin chub, and Cape Fear shiner. In this previous research, consistent test conditions were used (static acute toxicity tests, reconstituted ASTM hard water, and 60% dilution series) with test temperatures appropriate for the species and selected from the series identified by ASTM (1998).

MATERIALS AND METHODS

Test organisms

Hudson River American shad were obtained as fertilized eggs through the Pennsylvania Fish and Boat Commission, College Station, PA in cooperation with the Susquehanna River Restoration Program. Shad eggs were hatched and fry were cultured in well water (alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8, 18°C) at the Columbia Environmental Research Center (CERC, Columbia, MO). Shad were fed live brine shrimp nauplii and dried algae (*Spirulina*). Atlantic sturgeon, also of Hudson River origin, were obtained as fry from the USFWS, Northeast Fisheries Center, Lamar, PA as part of their hatchery evaluation program. Toxicity tests were also conducted with shortnose sturgeon as a surrogate for Atlantic sturgeon. Shortnose sturgeon were obtained from USFWS, Warm Springs Fish Technology Center, Warm Springs, GA. Sturgeon were held in well water for about one week before testing.

Before the start of a toxicity test, organisms were acclimated for a total of 96 h (USEPA 1975, ASTM 1998). For the first 48 h, organisms were acclimated to the test water (alkalinity 110 to120 mg/L as $CaCO_3$, hardness 160 to180 mg/L as $CaCO_3$; ASTM 1998) and temperature. The test organisms were then moved to other containers and held for an additional 48 h at the test temperature in 100% test water. Organisms were not fed during the 48 h of holding in 100% test water.

Chemicals

The chemicals used in testing were carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin (Table 1.1). Chemicals were selected to represent different classes of chemical and modes of toxic action. Organic chemical stock solutions were prepared by dissolving the chemical in reagent grade acetone, whereas stock solutions for copper were prepared by dissolving copper sulfate in deionized water. The maximum acetone concentration in any test container was 0.05 mL/L.

Organic and inorganic chemical stocks were analyzed to confirm nominal concentrations. Organic chemical analysis was conducted at ABC Laboratories (Columbia, MO) using gas chromatography. Copper stocks were confirmed at the CERC by atomic absorption spectrophotometry. Overall, the mean percent nominal stock concentration was 105%, with a range of 88% (permethrin, Table 1.1) to 131% (4-nonylphenol).

Toxicity tests

Static acute toxicity tests were conducted in basic accordance with procedures described in USEPA (1975) and ASTM (1998). Sturgeon exposures were conducted in 19.6-L glass jars containing 15 L of test solution, while shad exposures were conducted in 3.8-L glass jars containing 3 L of test solution. Test water was reconstituted hard water (ASTM 1998). Tests were conducted under ambient lighting.

The exposure series consisted of six concentrations with a 60% dilution series tested in triplicate. When a solvent was used, both a solvent control (0.05 mL/L) and a dilution water control were included for each species. Individual test series were randomly assigned to a waterbath and location within a waterbath (complete block design).

Fishes were counted into two groups (3 to 5 organisms per group) and pooled for each exposure replicate (7 to 10 organisms/replicate depending on average weight of fish). Mortality was the endpoint measured at 6, 12, 24, 48, 72, and 96 h of exposure and was defined as the lack of movement for a 5-s observation with the unaided eye. Dead animals were removed at each observational time. The study design is summarized in Table 1.2.

Water quality

Alkalinity, hardness, and pH were measured on each batch of reconstituted water before the start of the exposures. The pH was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 96 h of exposure. Dissolved oxygen was measured on the control, low, medium, and high exposure concentrations at 0 h and in those same treatments if organisms survived to 48 and 96 h of exposure. Tests with American shad had an average pH of 8.6 (range 8.1 to 8.8) and an average dissolved oxygen concentration of 8.5 mg/L (range 8.0 to 9.2). Atlantic sturgeon and shortnose sturgeon tests had an average pH of 8.4 (range 7.8 to 8.6). Dissolved oxygen concentrations for the tests with Atlantic sturgeon averaged 8.6 mg/L (range 4.8 to 9.4) while tests with shortnose sturgeon averaged 8.7 (range 5.2 to 9.1).

Statistical analysis

The LC50 and 95% confidence interval for each test was usually calculated using probit analysis. However, when probit analysis was not appropriate (i.e., less than two partial mortalities), LC50s and confidence intervals were calculated using moving average or a nonlinear interpolative procedure (Stephan 1977). The LC50s and confidence intervals were determined using nominal concentrations and not corrected for measured stock concentrations.

RESULTS AND DISCUSSION

Toxicity tests with American shad were attempted in 1997 and 1998. During 1997 control survival at 48 h of exposure was 80% in the water-only control and 75% in the acetone control (Table 1.3). This low control survival is of concern since it is less than that which is normally considered as acceptable in an acute test (90%; ASTM, 1998). Also, at 96 h of exposure with American shad, the water-only survival was 43% and the acetone control survival was 55%. Results at 96 h are considered unacceptable. We chose to present data for American shad based on 48-h exposures. However, conclusions regarding the chemical sensitivity of the American shad obtained at 48 h of exposure need to be carefully evaluated since these organisms were clearly stressed as indicated by the 48- and 96-h control results. During 1998, we cultured the American shad one month longer with the assumption that older organisms may provide acceptable control survival during the 96-h toxicity test. However, in 1998, during acclimation to the test waters, there was substantive mortality of American shad. In 1999, we planned to conduct toxicity tests for a third season, however there were only enough organisms obtained to conduct tests reported in Chapter 2.

In the toxicity tests with Atlantic sturgeon, control survival was 100% in both the water-only and acetone controls (Table 1.3). At 96 h of exposure, the acetone control had a control survival of 70%, while the water-only control survival was 100%. Mortality in the acetone control was due to all fish dying in one replicate. We have observed in other tests with sturgeon, in both exposure treatments and controls, that if a few fish die in a replicate, the water quickly fouls and most or all of the fish then die in that replicate. These observations indicate that conclusions regarding the chemical sensitivity of the sturgeon will need to be carefully evaluated. However, because the control survival was acceptable at 48 h of exposure, and mortality was only observed in one control replicate at 96 h of exposure, we have included results for the Atlantic sturgeon in this report. Control survival for both the water-only and acetone controls in toxicity tests with the shortnose sturgeon was 100% at both 48 and 96 h of exposure (Table 1.3).

Tables 1.4 to 1.9 summarize the 48- and 96-h LC50s for all five chemicals and each species. In general, at 96-h of exposure, permethrin was the most toxic compound and carbaryl was the least toxic compound. These results were similar to those reported in a previous study (USEPA 1995) with these five chemicals. The two phenolic compounds (4-nonylphenol and pentachlorophenol) and copper had LC50s in a similar range of concentrations.

For the following discussion, we have included data from the present study and data generated in previous cooperative research conducted between the USEPA (1995), USFWS, and USGS for the same five chemicals with rainbow trout, fathead minnows and 13 different threatened and endangered species - Apache trout, Lahontan cutthroat trout, greenback cutthroat trout, bonytail chub, Colorado squawfish, razorback sucker, fountain darter, greenthroat darter, shovelnose sturgeon, gila topminnow, boreal toad, spotfin chub, and Cape Fear shiner (Table 1.5 to 1.9). In this previous study, consistent test conditions were used (static acute toxicity tests, reconstituted ASTM hard water, and 60% dilution series) with test temperatures appropriate for the species and selected from the series specified by ASTM (1998).

After 48 h of exposure to carbaryl, copper, pentachlorophenol and permethrin, the LC50s for the American shad are lower than the 48-h LC50s for the standard test organisms, rainbow trout and fathead minnow (Table 1.4). However, the confidence intervals for tests with American shad and rainbow trout for copper and permethrin overlap, indicating that the difference between American shad and rainbow trout may not be significant. Except for exposures to carbaryl at 48 h of exposure, Atlantic sturgeon and shortnose sturgeon exhibited a similar sensitivity to chemical exposure. The 48-h LC50s for the shortnose and Atlantic sturgeon exposed to copper and pentachlorophenol were similar to the LC50s for rainbow trout. The two sturgeon species were more sensitive to 4-nonylphenol and permethrin than rainbow trout. While the Atlantic sturgeon and rainbow trout seem to be similar in sensitivity to carbaryl exposure, the shortnose sturgeon appears to be less sensitive to carbaryl. Collectively, these results indicate that the American shad, Atlantic and shortnose sturgeon are generally similar to or slightly more sensitive than the rainbow trout. As previously stated, the results for the American shad should be interpreted with caution given the high control mortality after 48 h of exposure.

In order to evaluate species sensitivity, within a chemical, we ranked 96-h LC50s for each species, including the shortnose and Atlantic sturgeon, from 1 (high sensitivity - low LC50) up to 16 (low sensitivity - high LC50). American shad were not included in this analysis due to high control mortality at 96 h of exposure. Ranks were then averaged across chemicals for each species (Table 1.10). The Atlantic sturgeon and shortnose sturgeon were the two most sensitive species of the 17 species evaluated in this comparison. Rainbow trout were the seventh most sensitive species.

In addition to relative species sensitivity, the magnitude of difference between LC50s is also important. Using data from the previous study for the six rainbow trout tests with each chemical (USEPA 1995), we calculated two factors (lowest 96-h LC50 / mean 96-h LC50; mean 96-h LC50 / highest 96-h LC50) which encompassed the range of LC50s for that chemical. For example, for the six toxicity tests conducted with rainbow trout and carbaryl (USEPA 1995), the lowest 96-h LC50 was 1.22 mg/L, the highest 96-h LC50 was 3.11, and the mean 96-h LC50 was 1.88 mg/L. Factors calculated for rainbow trout carbaryl exposures were 0.60 and 0.65 with a geometric mean of 0.62. For the five chemicals tested with rainbow trout, the geometric mean factor for all five chemicals was 0.69 with a range of 0.60 (permethrin) to 0.80 (pentachlorophenol). We followed the same procedure for fathead minnows and the five chemicals. Fathead minnows had a geometric mean factor for the five chemicals of 0.65 with a

range of 0.57 (pentachlorophenol) to 0.73 (permethrin). If a factor of 0.67 (geometric mean of rainbow trout and fathead minnow) is selected as representative of the normal range in LC50 (expected range = [LC50 X 0.67] to [LC50 / 0.67]) for a specific chemical and species, then the sensitivities of listed species can be evaluated in terms of how often 96-h LC50s for the listed species differed by more than a factor of 0.67 from the 96-h LC50 for either rainbow trout or fathead minnows.

For all possible comparisons of shortnose and Atlantic sturgeon to the range of 96-h LC50s that might be expected for fathead minnows (n=9), the two sturgeon species have LC50s less than the expected range. When the comparison is made to rainbow trout (n=9), the two sturgeon species have six 96-h LC50s less than the expected range of LC50s for rainbow trout (Tables 1.5 to 1.9).

A final evaluation would be to determine the greatest difference between the 96-h LC50s of the rainbow trout and Atlantic sturgeon and shortnose sturgeon. Within a chemical, we compared the lowest 96-h LC50 for either sturgeon to the geometric mean 96-h LC50 for rainbow trout. For Atlantic sturgeon, we were able to compare only two tests, copper and 4-nonylphenol. For copper the factor was 0.8 while for 4-nonylphenol the factor was 0.3. We were able to calculate a factor with shortnose sturgeon for carbaryl, copper, 4-nonylphenol and pentachlorophenol. The average factor for those four tests was 0.7 with a range of 0.4 to 1.0.

Overall, these assessments would indicate that sturgeon are similar to or somewhat more sensitive to contaminant exposure than rainbow trout. These results are similar to acute toxicity results obtained by the U.S. Fish and Wildlife Service, Northeast Fishery Center, Lamar, PA (Kim King, personal communication). In those studies, Atlantic sturgeon were exposed for 96 h to formalin, chloramine-T, and sodium chloride. The 96-h LC50s for Atlantic sturgeon exposed to formalin, chloramine-T or sodium chloride were similar to the LC50s obtained for the most sensitive fish species (striped bass, rainbow trout) exposed to these same chemicals.

Because of the difficulty in testing with sturgeon, conclusions regarding the chemical sensitivity of the sturgeon need to be interpreted with caution. If sturgeon are more sensitive than rainbow trout, then a factor could be used to estimate an LC50 for sturgeon from rainbow trout data. The most conservative approach would be to use the factor of 0.3 determined with Atlantic sturgeon exposed to 4-nonylphenol. Expected environmental concentrations (e.g., water quality criteria, pesticides) could be compared to this calculated LC50 and determinations if the Atlantic sturgeon is at risk could be made.

Table 1.1 Source and percent active ingredient of chemicals used in toxicity tests.

Chemical	Source	Active Ingredient (%)	Chemical Stock Confirmation (% of Nominal)	Use	Mode of Action
Carbaryl	Donated by Rhone-Pôulenc Agricultural Co., Research Triangle Park, NC	99.7	117	carbamate insecticide	inhibitor of cholinesterase activity
Copper sulfate	Fisher Chemical, St. Louis, MO	25.5	94	mining, industrial, fungicide	interferes in osmoregulation
4-nonylphenol	Fluka Chemical, New York, NY	85.0	131	nonylphenol ethoxylate detergents	narcotic and oxidative stressor
Pentachlorophenol	Aldrich Chemical, Milwaukee, WI	99.0	97	wood preservative, molluscicide	uncoupler of oxidative phosphorylation (i.e. interferes with cellular energetics)
Permethrin	Donated by ICI Americas Inc., Richmond, CA	95.2	88	pyrethroid insecticide	neurotoxin

Table 1.2 Summary of study design for the comparative toxicity of selected chemicals to listed species.

Test type:	Static acute
Test volume:	Shortnose sturgeon - 15 L Atlantic sturgeon - 15 L American shad - 3 L
Test temperature:	Shortnose sturgeon - 17°C Atlantic sturgeon - 17°C American shad - 22°C
Water Quality:	Reconstituted ASTM hard (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$)
Chemicals:	Carbaryl, copper, 4-nonylphenol, pentachlorophenol, permethrin
Dilution series:	60%
Replicates/number of organisms per replicate:	Shortnose sturgeon - 3 replicates/7 fish per replicate Atlantic sturgeon - 3 replicates/9 fish per replicate American shad - 3 replicates/10 fish per replicate
Average weight:	Shortnose sturgeon - 0.74 g (wet wt) Atlantic sturgeon - 1.11 g (wet wt) American shad - 0.006 g (dry wt)
Observations:	Mortality at 6, 12, 24, 48, 72, and 96 h of exposure

Table 1.3 Summary of control survival at 48 and 96 h of exposure for American shad, Atlantic sturgeon, and shortnose sturgeon.

Species	Control type	Exposure time	
		48 hours	96 hours
American shad	water	80	43
	acetone	75	55
Atlantic sturgeon	water	100	100
	acetone	100	70
Shortnose sturgeon	water	100	100
	acetone	100	100

Table 1.4 Calculated 48-h LC50 and confidence interval (parentheses) for American shad, Atlantic sturgeon and shortnose sturgeon. Also included is the 48-h LC50 for rainbow trout and fathead minnow (USEPA 1995). For rainbow trout and fathead minnow the numbers in parentheses are the range of LC50s (n=6) for that species as reported in USEPA (1995) using similar testing conditions.

Species	Chemical				
	Carbaryl	Copper	4-nonylphenol	Pentachlorophenol	Permethrin
American shad	<0.08	0.05 (0.04 - 0.06)	0.05 (0.04-0.06)	0.04 (0.03 - 0.05)	2.08 (1.78 - 2.37)
Atlantic sturgeon	1.28 (1.06 - 1.50)	0.15 (0.09 - 0.24)	0.08 (0.06 - 0.11)	0.19 (0.17 - 0.22)	<1.2
Shortnose sturgeon	4.23 (3.60 - 6.00)	0.15 (0.13 - 0.18)	0.08 (0.06 - 0.11)	0.16 (0.13 - 0.18)	<1.2
Rainbow trout	2.45 (1.27 - 3.50)	0.09 (0.06 - 0.17)	0.22 (0.17 - 0.27)	0.15 (0.11 - 0.19)	3.49 (1.65 - 6.00)
Fathead minnow	7.9 (1.88 - 10.00)	0.66 (0.50 - 1.16)	0.29 (0.17 - 0.40)	0.28 (0.14 - 0.50)	10.1 (8.55 - 16.8)

Table 1.5 Acute toxicity of carbaryl (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. In addition, a factor is calculated which relates the geometric mean LC50 for rainbow trout (n=6, USEPA 1995) to the LC50 for all other individual species.

Species	Carbaryl				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	1.88	5	-	1.0	X< >
Fathead minnow	5.21	14	< >X	2.8	-
Apache trout	1.54	2	<X>	0.8	X< >
Greenback cutthroat trout	1.55	3	<X>	0.8	X< >
Lahontan cutthroat trout	2.25	8	<X>	1.2	X< >
Bonytail chub	3.49	11	< >X	1.9	X< >
Colorado squawfish	3.07	9	< >X	1.6	X< >
Razorback sucker	4.35	12	< >X	2.3	<X>
Fountain darter	2.02	6	<X>	1.1	X< >
Greenthroat darter	2.14	7	<X>	1.1	X< >
Shovelnose sturgeon	nc[1]	-	-	-	-
Gila topminnow	>3.0	nr[2]	-	nc	-
Boreal toad	12.3	15	< >X	6.5	< >X
Shortnose sturgeon	1.81	4	<X>	1.0	X< >
Spotfin chub	3.41	10	< >X	1.8	X< >
Cape Fear shiner	4.51	13	< >X	2.4	<X>
Atlantic sturgeon	<0.8	1	X< >	-	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 1.6 Acute toxicity of copper (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. In addition, a factor is calculated which relates the geometric mean LC50 for rainbow trout (n=6, USEPA 1995) to the LC50 for all other individual species.

Species	Copper				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	0.08	5.5	-	1.0	X< >
Fathead minnow	0.47	16	< >X	5.9	-
Apache trout	0.07	3.5	<X>	0.9	X< >
Greenback cutthroat trout	>0.03	nr[2]	-	-	-
Lahontan cutthroat trout	0.07	3.5	<X>	0.9	X< >
Bonytail chub	0.22	12	< >X	2.8	X< >
Colorado squawfish	0.43	15	< >X	5.4	<X>
Razorback sucker	0.27	14	< >X	3.4	X< >
Fountain darter	0.06	1.5	<X>	0.8	X< >
Greenthroat darter	0.26	13	< >X	3.3	X< >
Shovelnose sturgeon	0.16	10.5	< >X	2.0	X< >
Gila topminnow	0.16	10.5	< >X	2.0	X< >
Boreal toad	0.12	9	<X>	1.5	X< >
Shortnose sturgeon	0.08	5.5	<X>	1.0	X< >
Spotfin chub	0.09	7	<X>	1.1	X< >
Cape Fear shiner	0.11	8	<X>	1.4	X< >
Atlantic sturgeon	0.06	1.5	<X>	0.8	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 1.7 Acute toxicity of 4-nonylphenol (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. In addition, a factor is calculated which relates the geometric mean LC50 for rainbow trout (n=6, USEPA 1995) to the LC50 for all other individual species.

Species	4-Nonylphenol				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	0.19	11.5	-	1.0	<X>
Fathead minnow	0.27	15	<X>	1.4	-
Apache trout	0.17	8.5	<X>	0.9	X< >
Greenback cutthroat trout	0.15	7	<X>	0.8	X< >
Lahontan cutthroat trout	0.18	10	<X>	0.9	<X>
Bonytail chub	0.29	16	< >X	1.5	<X>
Colorado squawfish	0.26	14	<X>	1.4	<X>
Razorback sucker	0.17	8.5	<X>	0.9	X< >
Fountain darter	0.11	4	X< >	0.6	X< >
Greenthroat darter	0.19	11.5	<X>	1.0	<X>
Shovelnose sturgeon	<0.13	nr[2]	-	-	X< >
Gila topminnow	0.23	13	<X>	1.2	<X>
Boreal toad	0.12	5	X< >	0.6	X< >
Shortnose sturgeon	0.08	2.5	X< >	0.4	X< >
Spotfin chub	0.08	2.5	X< >	0.4	X< >
Cape Fear shiner	0.14	6	<X>	0.7	X< >
Atlantic sturgeon	0.05	1	X< >	0.3	X< >

[1]nc - not calculated
[2]nr - not ranked

Table 1.8 Acute toxicity of pentachlorophenol (mg/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. In addition, a factor is calculated which relates the geometric mean LC50 for rainbow trout (n=6, USEPA 1995) to the LC50 for all other individual species.

Species	Pentachlorophenol				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	0.16	4	-	1.0	<X>
Fathead minnow	0.25	10	<>X	1.6	-
Apache trout	0.11	2.5	<X>	0.7	<X>
Greenback cutthroat trout	>0.01	nr[2]	-	-	-
Lahontan cutthroat trout	0.17	5	<X>	1.1	<X>
Bonytail chub	0.23	8	<X>	1.4	<X>
Colorado squawfish	0.24	9	<X>	1.5	<X>
Razorback sucker	0.28	12	<>X	1.8	<X>
Fountain darter	0.11	2.5	<X>	0.7	X<>
Greenthroat darter	0.18	6	<X>	1.1	<X>
Shovelnose sturgeon	nc[1]	-	-	-	-
Gila topminnow	0.34	13	<>X	2.1	<X>
Boreal toad	0.37	14	<>X	2.3	<X>
Shortnose sturgeon	0.07	1	X<>	0.4	X<>
Spotfin chub	0.26	11	<>X	1.6	<X>
Cape Fear shiner	0.19	7	<X>	1.2	<X>
Atlantic sturgeon	<0.04	nr	-	-	-

[1]nc - not calculated
[2]nr - not ranked

Table 1.9 Acute toxicity of permethrin (ug/L) to 16 fishes and one amphibian. Data includes the 96-h LC50 and the relative species rank sensitivity. Also included is an assessment to determine if the 96-h LC50 for a particular species is out of the average range of LC50s for either rainbow trout or fathead minnows. In addition, a factor is calculated which relates the geometric mean LC50 for rainbow trout (n=6, USEPA 1995) to the LC50 for all other individual species.

Species	Permethrin				
	LC50	Rank	RBT <0.67>	RBT LC50 Ratio	FHM <0.67>
Rainbow trout	3.31	7	-	1.0	X<>
Fathead minnow	9.38	11	<>X	2.8	-
Apache trout	1.71	5	X<>	0.5	X<>
Greenback cutthroat trout	>1.0	nr	-	-	-
Lahontan cutthroat trout	1.58	3	X<>	0.5	X<>
Bonytail chub	>25.0	13	<>X	-	<>X
Colorado squawfish	24.4	12	<>X	7.4	<>X
Razorback sucker	5.95	10	<>X	1.8	X<>
Fountain darter	3.34	8	<X>	1.0	X<>
Greenthroat darter	2.71	6	<X>	0.8	X<>
Shovelnose sturgeon	nc[1]	nr[2]	-	-	-
Gila topminnow	>10.0	nr	<>X	-	-
Boreal toad	>10.0	nr	<>X	-	-
Shortnose sturgeon	<1.2	1.5	X<>	-	X<>
Spotfin chub	1.70	4	X<>	0.5	X<>
Cape Fear shiner	4.16	9	<X>	1.3	X<>
Atlantic sturgeon	<1.2	1.5	X<>	-	X<>

[1]nc - not calculated
[2]nr - not ranked

Table 1.10 Summary rank for 16 fishes and one amphibian. The summary rank was calculated by averaging the individual ranks obtained for each species (Tables 1.5 to 1.9) within a chemical and then reranking.

Family	Species	Summary rank
Salmonidae	Rainbow trout	7
Cyprinidae	Fathead minnow	17
Salmonidae	Apache trout	3
Salmonidae	Greenback cutthroat trout	5
Salmonidae	Lahontan cutthroat trout	6
Cyprinidae	Bonytail chub	15
Cyprinidae	Colorado squawfish	14
Catostomidae	Razorback sucker	13
Percidae	Fountain darter	4
Percidae	Greenthroat darter	10
Acipenseridae	Shovelnose sturgeon	11
Poeciliidae	Gila topminnow	16
Bufonidae	Boreal toad	12
Acipenseridae	Shortnose sturgeon	2
Cyprinidae	Spotfin chub	8
Cyprinidae	Cape Fear shiner	9
Acipenseridae	Atlantic sturgeon	1

Chapter 2: Evaluation of the use of standard effluent toxicity tests for protection of American shad and Atlantic sturgeon.

INTRODUCTION

The U.S. Clean Water Act (CWA) specifies "it is the national policy that the discharge of toxic pollutants in toxic amounts be prohibited" (Section 101(a)(3)). The CWA provides an integrated approach to protection of aquatic ecosystems through the development of water quality criteria and the control of toxic discharges (National Pollutant Discharge Elimination System - NPDES; 45 FR 33520). Programs designed to provide protection of freshwater aquatic environments from toxic discharges commonly include whole-effluent toxicity tests with the cladoceran *Ceriodaphnia dubia*, fathead minnow (*Pimephales promelas*), and algae (*Selenastrum capricornutum*). The assumption is that results of toxicity tests using these test species are protective of effects on other organisms including endangered and threatened (listed) species.

Surrogate species, such as cladocerans and fathead minnows, are the typical freshwater organisms used in standardized tests (USEPA 1993). However, it is unknown if the sensitivities of these species to contaminant exposure represent the sensitivities of listed species. Reports on biological surveys of streams and rivers in states such as Ohio have suggested that effluent test protocols using standard procedures might not adequately protect aquatic ecosystems (Yoder 1989). NPDES permits often require toxicity tests with effluents using embryo-larval fathead minnows and *Ceriodaphnia dubia*. The objective of the present study was to determine the degree of protection afforded American shad and Atlantic sturgeon through the use of standard species in whole-effluent toxicity tests.

Ninety-six hour water-renewal toxicity tests were conducted using standard effluent test procedures (USEPA 1993). We conducted 96-h survival studies with embryo-larval fathead minnows and analogous exposures using the listed species. Effects on survival of *C. dubia* were also evaluated. Tests were conducted with two effluents collected from discharges into the Hudson River, New York. One effluent was from a combined industrial and residential wastewater treatment plant without disinfection. The other effluent was from a primarily residential wastewater treatment plant with chlorination used as disinfection.

MATERIALS AND METHODS

Test organisms

Hudson River American shad were obtained as fertilized eggs through the Pennsylvania Fish and Boat Commission, College Station, PA in cooperation with the Susquehanna River Restoration Program. Atlantic sturgeon, also of Hudson River origin, were obtained as fry from the USFWS, Northeast Fisheries Center, Lamar, PA as part of their hatchery evaluation program. Fathead minnows were obtained from Aquatic Biosystems, Inc., Fort Collins, CO and *C. dubia*

were obtained from Columbia Environmental Research Center (CERC) cultures. All fish larvae or eggs were held in well water (18°C, alkalinity 258 mg/L as $CaCO_3$, hardness 286 mg/L as $CaCO_3$, pH 7.8) until testing began.

Fathead minnows and American shad tests were started with fish less than 24-h old. Tests with Atlantic sturgeon were conducted with fish less than 48-h old. *Ceriodaphnia dubia* were cultured in ASTM hard water (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$; ASTM 1998) and tested when less than 24-h old.

Effluents

Samples were collected from two effluents (designated E-1 and E-2) that discharge into the Hudson River, New York. Grab samples (20 to 40 L) were collected from each effluent on May 4 and 6, 1999 for the Atlantic sturgeon and May 31 and June 1, 1999 for the shad exposures. Samples were shipped on ice to CERC for toxicity testing and chemical analysis. Samples for the sturgeon were tested immediately after receipt. Effluents to be tested with the American shad had to be stored seven days prior to testing. Effluents are typically not stored for this length of time prior to testing. However, it was difficult to predict the day of hatch for the American shad. For that reason, effluents were obtained and then stored until the shad hatched.

Toxicity tests

We attempted to conduct 96-h acute toxicity tests following EPA procedures described for effluents (USEPA 1993). Toxicity test procedures are summarized in Table 2.1. Effluents were tested in a 50% dilution series (100, 50 25, 12.5 and 6.25% effluent). Dilution water was reconstituted ASTM hard water (alkalinity 110 to 120 mg/L as $CaCO_3$, hardness 160 to 180 mg/L as $CaCO_3$; ASTM 1998). American shad tests were conducted at the standard effluent procedure temperature (25°C) while Atlantic sturgeon were tested at a more appropriate environmental temperature (20°C). Fathead minnow and *Ceriodaphnia dubia* tests conducted concurrently with American shad and Atlantic sturgeon, were tested at the temperatures used for the American shad and Atlantic sturgeon tests. Because of age requirements for the fish species at the start of the test (<24 or <48 h old), none of the fish were acclimated to the test water before starting toxicity tests. However, *C. dubia* were cultured in this reconstituted hard water. The test was conducted in ambient light with 16 hours of light and 8 hours of dark.

For toxicity tests with fish, each exposure concentration was tested in triplicate. Fish were counted into groups of five with two groups pooled for each exposure replicate (10 fish per 1 L beaker - for a total of 30 fish/treatment). American shad were tested in 250-ml beakers which contained 200 ml of test solution. Atlantic sturgeon were tested in 3.8-L jars which contained 3.5 L of test solution. Water was renewed every 24 hours of the exposure.

Ten *C. dubia* were tested in individual 30 mL beakers containing 15 mL of test solution with one animal per beaker. Survival was determined daily for *C. dubia*. Water was renewed every 24 hours of the exposure.

Elements were measured on unfiltered effluent samples. Elements were determined at CERC using a semi-quantitative scan by ICP-MS. Table 2.2 is a summary of the elements measured in the two effluents for each sampling period. In addition, ammonia and chlorine were measured on each effluent sample. Total ammonia concentrations (mg/L N) were measured with an Orion EA940 Expandable ionAnalyzer, and Orion 95-12 ammonia electrode. For tests conducted with American shad, total ammonia was 11.2 mg/L for E-1 and 12.4 for E-2. For those same effluents sampled later and used in toxicity tests with Atlantic sturgeon, total ammonia was 17 mg/L for E-1 and 9.0 mg/l for E-2. Chlorine was measured using a Fisher and Porter amperometric titrator. Chlorine was not detected in any samples (minimum detection limit, 5 ug/L). Dissolved oxygen, temperature, and pH were measured on the control, low, medium, and high exposure concentrations daily in the fresh test solution and in the test solution after 24 h of exposure. For tests with American shad, E-1 had an average pH of 8.2 (range 8.0 to 8.5) and an average dissolved oxygen concentration of 8.3 mg/L (range 8.0 to 8.8). Effluent sample E-2, used in tests with American shad, had an average pH of 8.3 (range 8.0 to 8.8) and an average dissolved oxygen concentration of 8.2 mg/L (range 7.7 to 8.8). For tests with Atlantic sturgeon, E-1 had an average pH of 8.3 (range 7.9 to 8.5) and an average dissolved oxygen concentration of 8.0 mg/L (range 6.8 to 8.9). Effluent sample E-2, used in tests with American shad, had an average pH of 8.3 (range 7.9 to 8.5) and an average dissolved oxygen concentration of 8.3 mg/L (range 6.8 to 9.2).

The purpose of this study was to evaluate the relative response of American shad and Atlantic sturgeon compared to fathead minnows and *C. dubia*. For that reason, no attempt has been made to identify any toxic components of the effluents. However, the concentration of total ammonia in effluent E-1 and E-2 appears to be elevated. Additionally, the copper concentration in effluent E-2 is somewhat elevated. Additional evaluations should be conducted to determine if the concentrations of these effluent components meet appropriate water quality standards.

Statistical analysis

The LC50 and 95% confidence interval for each test was usually calculated using probit analysis. However, when probit analysis was not appropriate (i.e., less than two partial mortalities), LC50s and confidence intervals were calculated using moving average or a nonlinear interpolative procedure (Stephan 1977). The LC50s and confidence intervals were determined using nominal concentrations

RESULTS AND DISCUSSION

American shad and Atlantic sturgeon did not respond well to the test procedures used in this study and results from this study should be interpreted cautiously. Tables 2.3 and 2.4 summarize for each species, the control mortality and the percent mortality for each dilution within a test. Because of the excessive control mortality for both American shad and Atlantic sturgeon, we considered a test acceptable if there were about 70% control survival (about 30% control mortality).

American shad control mortality was 70 and 72.5 % at 48 hours of exposure (Table 2.3). The test for American shad, fathead minnows and *C. dubia* was ended after 48 hours of exposure because of the excessive control mortality for American shad. At 24 h of exposure, the control mortality for American shad was 12.5 and 30%. For both effluents, and in all exposure concentrations there was a mortality of shad consistent with that found in the controls with a range of 12.5 to 40%. For E-1, shad mortality ranged from 12.5 to 40% (Table 2.3). For E-2 mortality of shad ranged from 12.5 to 27.5% (Table 2.3). The 40% mortality of shad for E-1 was measured in the 50% effluent dilution, however, the 100% effluent sample had a mortality of 20%, so we do not believe that the mortality of 40% exhibited in the E-1 50% effluent was toxicologically significant. Fathead minnows exhibited no consistent toxic response to either effluent during the first 24 h of exposure. If the 24-h toxicity results for both the fathead minnow and the American shad are considered acceptable, then it could be concluded that the two fish species exhibit a similar toxicological response. This is further supported if the 48-h data is considered. While overall mortality is high for the American shad, at 48-h of exposure, the fathead minnow exhibits a very slight and probably biologically insignificant increase in toxicity in the E-1 50 and 100% dilution concentrations. The American shad in those two concentrations exhibited 100% mortality. It should be kept in mind that across the controls and lower exposure concentrations, mortality for the American shad was 62.5 to 70%. Similarly a wide range of mortality (47.5 to 70%) was observed for American shad across all concentrations for E-2 at 48-h of exposure. The fathead minnow exhibits no consistent toxicity at 48-h of exposure to E-2. This would also indicate that the fathead minnow and American shad are exhibiting a similar toxicological response. *C. dubia* responses during this test are unacceptable (100% mortality in well water control) and therefore have not been used in the species comparison for the American shad.

Table 2.4 summarizes the toxicity data for the Atlantic sturgeon. As was the case for the American shad, control mortality for the Atlantic sturgeon was about 30% in the first 24 h of exposure. Fathead minnows and *C. dubia* exhibit a similar toxic response to E-1 (an increase in mortality at the 100% effluent concentration). Atlantic sturgeon exhibit only a slight increase in mortality at the 100% effluent concentration after 24 h of exposure.

The results of exposure to E-2 are markedly different from the exposure results to E-1. After 24-h of exposure to 100% E-2, *C. dubia* exhibited 50% mortality and the fathead minnow had no mortality. However, Atlantic sturgeon exhibits complete mortality (100%) in the E-2 100% effluent concentration. Additionally, 55% of the sturgeon (an increase of 25% over the accepted background of 30%) died with exposure to 50% E-2 effluent. As the exposure continued (48 h and 96 h results) the fathead minnow began to exhibit a toxic response in the E-2 100% effluent. While these results might indicate that the Atlantic sturgeon is more sensitive to exposure than the fathead minnow, it is more likely that the observed response indicates that the Atlantic sturgeon were already stressed. Fathead minnows may have been able to handle the test conditions and therefore, the response to contaminant exposure occurs at a later time period.

In summary, neither the American shad nor the Atlantic sturgeon were suited for the testing procedure used in this study. If these species are proposed for testing, extensive method development would be required. Factors such as handling procedures, optimum feeding rates,

optimum test temperature, expected test to test variation and expected control survival would need to be established. From the results obtained in this study, the fathead minnow survival test appears to be a reliable estimator of effects to American shad and Atlantic sturgeon. However, given the difficulty in testing these species, results from this study should be interpreted cautiously.

Table 2.1 Summary of study design for the comparative toxicity of effluents to American shad and Atlantic sturgeon.

Test type:	Daily renewal
Test temperature:	American shad - 25°C Fathead minnow-25°C *C. dubia* - 25°C Atlantic sturgeon - 20°C Fathead minnow-20°C *C. dubia* - 20°C
Dilution water:	Reconstituted ASTM hard
Effluents:	E-1 and E-2
Test container:	Fathead minnow and American shad - 250 ml beaker with 200 ml test solution Atlantic sturgeon - 3.785 L jar with 3.5 L test solution *C. dubia* - 30 ml beaker with 15 ml test solution
Dilution series:	50%
Age of organisms:	Fathead minnow, American shad and *C. dubia* < 24 h old Atlantic sturgeon < 48 h old
Observations:	Mortality every 24 h for 96 h

Table 2.2 Concentrations of elements (ug/L) from effluents (E-1 and E-2) tested with Atlantic sturgeon (ATS) and American shad (AMS). Elements were reported if one sample had greater than the minimum detectable concentration for that element. For each species, the same effluent was sampled but at different times.

Element	ATS		AMS		Element	ATS		AMS	
	E-1	E-2	E-1	E-2		E-1	E-2	E-1	E-2
Li	10	6	10	6	Rb	5	7	5	6
B	100	100	100	100	Sr	300	200	300	200
Na	100000	100000	100000	100000	Zr	2	4	<1	2
Mg	10000	9000	10000	9000	Mo	30	1	20	10
Al	200	600	40	100	Ag	0.4	5	0.2	2
K	6000	7000	5000	8000	Cd	6	0.3	6	0.2
Ca	60000	50000	60000	50000	Sn	2	5	2	0.9
Ti	10	50	2	20	Sb	0.6	0.6	0.5	0.5
V	1	2	2	0.9	I	200	9	200	5
Cr	2	2	2	1	Ba	60	40	70	30
Mn	200	200	300	200	La	0.1	0.3	<0.1	0.1
Fe	200	500	<1	400	Ce	0.1	0.5	<0.1	0.1
Co	1	0.4	0.9	0.3	Nd	0.1	0.3	<0.1	0.1
Ni	2	3	1	3	Hf	0.1	0.1	<0.1	0.1
Cu	10	60	6	40	Ta	<0.1	0.1	<0.1	0.1
Zn	40	60	20	50	W	0.1	0.1	<0.1	<0.1
Ga	0.1	0.4	0.1	0.2	Au	0.4	0.2	0.1	2
As	4	1.0	2.0	1.0	Pb	2	6	<1	3
Br	200	200	200	200					

Table 2.3 Summary of mortality (%) for C. dubia, fathead minnow (FHM) and American shad (AMS) at 24 and 48 h of exposure.

Effluent	Time	Species	Well	ASTM	6.25	12.5	25	50	100	LC50
E-1	24	C. dubia	50	10	30	70	100	30	40	nc[1]
		FHM	0	0	0	0	0	2.5	7.5	>100
		AMS	12.5	30	17.8	20	12.5	40	20	>100
	48	C. dubia	100	10	30	70	100	30	40	nc
		FHM	0	0	0	0	0	2.5	10	>100
		AMS	70	72.5	69	67.5	62.5	100	100	nc
E-2	24	C. dubia	50	10	0	20	30	30	30	nc
		FHM	0	0	2.5	0	0	0	0	>100
		AMS	12.5	30	17	20	12.5	27.5	22.5	>100
	48	C. dubia	100	10	0	50	30	40	30	nc
		FHM	0	0	25	0	0	0	0	>100
		AMS	70	72.5	60	60	47.5	50	70	>100

nc[1] - not calculated

Table 2.4 Summary of mortality (%) for C. dubia, fathead minnow (FHM) and Atlantic sturgeon (ATS) at 24, 48 and 96 h of exposure.

Effluent	Time	Species	Well	ASTM	6.25	12.5	25	50	100	LC50
E-1	24	C. dubia	0	20	0	0	0	20	50	100
		FHM	0	2.5	2.5	5	0	5	15	>100
		ATS	31.6	21	22.5	27.5	12.5	23.1	37.1	>100
	48	C. dubia	10	30	0	0	10	20	60	85
		FHM	0	2.5	2.5	5	0	7.7	27.5	>100
		ATS	44.7	26.3	35	40	17.5	30	42.9	>100
	96	C. dubia	10	30	10	0	20	30	60	80
		FHM	5	15	5	2.5	2.5	10	27.5	>100
		ATS	54	33	37.5	45	22.5	31.8	48.6	>100
E-2	24	C. dubia	0	20	50	0	20	10	50	>100
		FHM	0	2.5	0	0	0	0	0	>100
		ATS	31.6	21	35	32.5	32.5	55	100	<100
	48	C. dubia	10	30	50	20	30	30	50	>100
		FHM	0	2.5	0	0	2.5	2.5	12.5	nc[1]
		ATS	44.7	26.3	52.5	45	67.5	90	100	nc
	96	C. dubia	10	30	50	30	30	40	60	nc
		FHM	5	15	2.5	2.5	7.5	2.5	22.5	>100
		ATS	54	33	90	97.5	95	95	100	nc

nc[1] - not calculated

CONCLUSIONS AND RECOMMENDATIONS

In the first assessment, acute toxicity tests (96-h LC50) were conducted with early life-stage American shad, Atlantic sturgeon, and shortnose sturgeon using five chemicals having different toxicological modes of action. Chemicals included carbaryl, copper, 4-nonylphenol, pentachlorophenol, and permethrin. These chemicals have been tested in previous cooperative research conducted between the U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, and U.S. Geological Survey for the same five chemicals with early life-stage rainbow trout, fathead minnows and 13 different threatened and endangered species. After 48 h of exposure to carbaryl, copper, pentachlorophenol and permethrin, the LC50s for the American shad were lower than the 48-h LC50s for the standard test organisms, rainbow trout and fathead minnow. However, the confidence intervals for tests with American shad and rainbow trout for tests with copper and permethrin overlap, indicating that the difference between American shad and rainbow trout may not be significant. The results for the American shad should be interpreted with caution given the high control mortality after 48 h of exposure. Results for tests conducted with the two species of sturgeon indicate that the sturgeon are somewhat more sensitive to contaminant exposure than are the rainbow trout. However, because of the difficulty also associated in testing sturgeon, conclusions regarding the chemical sensitivity of the sturgeon also need to be interpreted with caution. If sturgeon are more sensitive than rainbow trout, then a factor could be used to estimate an LC50 for sturgeon from rainbow trout data. The most conservative approach would be to use the factor of 0.3 determined with Atlantic sturgeon exposed to 4-nonylphenol. Expected environmental concentrations (e.g., water quality criteria, pesticides) could be compared to this calculated LC50 and determinations if the Atlantic sturgeon is at risk could be made.

For the second assessment, 96-h water-renewal toxicity tests were conducted using standard effluent test procedures. Attempts were made to conduct 96-h survival studies with embryo-larval fathead minnows and analogous exposures using American shad and Atlantic sturgeon. Effects on survival of *C. dubia* were also evaluated. Tests were conducted with two effluents collected from discharges into the Hudson River, New York. Neither the American shad nor the Atlantic sturgeon were suited for the testing procedure used in this study. If these species are proposed for testing, extensive method development would be required. Factors such as handling procedures, optimum feeding rates, optimum test temperature, expected test to test variation and expected survival would need to be established. From the results obtained in this study, the fathead minnow survival test appears to be a reliable estimator of effects to American shad and Atlantic sturgeon.

Given the difficulty in testing the Atlantic sturgeon and American shad, results from this study should be interpreted very cautiously. However, if used as a preliminary estimate of sensitivity to contaminant exposure, then the results from this study would indicate that these two species are sensitive to exposure to environmental contaminants when compared to other fish species including the surrogate species. Procedures and regulations which are protective of sensitive fish species (e.g., rainbow trout) or are protective of Atlantic sturgeon or American shad specifically, would likely need to be implemented. Prior to any changes being made in current regulatory procedures and regulations, results from this study should be verified by additional method development and testing with the American shad and Atlantic sturgeon.

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