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SUB-CHRONIC TOXICITY EVALUATION OF MAJOR POINT SOURCE DISCHARGERS IN THE GRAND CALUMET RIVER AND INDIANA HARBOR CANAL, INDIANA, USING THE EMBRYO-LARVAL SURVIVAL AND TERATOGENICITY TEST

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ABSTRACT: Preliminary and definitive use of sub-chronic embryo-larval toxicity testing was conducted to interpret the specific impact of 19 point source dischargers along the Grand Calumet River and Indiana Harbor Canal. Preliminary screens were utilized to evaluate potential effects in 100% effluent, when compared to the control. Hammond SD had a significant teratogenic response during the preliminary test but did not exhibit a reduction in larval survival. Six positive results were obtained and retested as definitive tests. USX company was unavailable for definitive testing due to plant shutdown. East Chicago Sanitary District did not elicite a chronic response during definitive testing. However, statistically significant teratogenic responses were observed in larvae in all dilutions tested. Inland Steel outfalls 008 and 014 had chronic values of 77.5% and 42.4% effluent, respectively. Only Inland Steel outfall 014 had a statistically significant teratogenic response, affecting larvae in all dilutions tested. E.I. DuPont de Nemours and Company within 168 hrs of exposure, concentrations above 30% effluent had significant morality. A chronic value of 17.3% effluent was calculated for DuPont and statistically significant teratogenic responses were observed in dilutions above 30% effluent. Hatchability was unaffected in all preliminary tests except USX outfall 034. Statistically significant differences in definitive test hatchability were observed in 100% Inland Steel outfalls 008 and 014.

INTRODUCTION

Water quality in the Calumet region of northwest Indiana and northeast Illinois has been a matter of public concern for more than a decade (U.S. Department of the Interior, 1966, 1967; U.S. Environmental Protection Agency, 1982). The watershed is heavily urbanized, with the principal cities of Gary, Hammond, East Chicago, and Whiting collectively supporting populations over 500,000. The basin also includes one of the most concentrated steel and petrochemical industrial complexes in the United States.

TABLE 1. Summary of test procedures utilized for embryo-larval survival and teratogenicity testing of Grand Calumet River Basin.

Test type	Static Renewal
Temperature	26° ± 1° C
Light quality	Ambient environmental chamber
Light intensity	50-100 ft-c
Photoperiod	16 hrs light, 8 hrs dark
Test chamber size	600 mL
Test solution volume	400 mL
Renewal of test solutions	Every 24 hrs
Age of test organisms	Less than 48 hrs old embryos
Number of embryos/chamber	50
Number of replicate test chambers per concentration	2
Embryos/concentration	100
Feeding regime	Feeding not required
Aeration	None, unless dissolved oxygen is below 40% saturation
Dilution water	Dechlorinated micro-filtered tapwater
Test concentrations	0, 3, 10, 30, 60, 100% effluent
Dilution	0.3
Test duration	192 hrs (8 days)
Measurement end-points	Percent hatchability; percent larval survival; relative percent teratogenicity of live and dead organisms

Alarm over the poor water quality in streams draining this region into southern Lake Michigan initiated the formation of the Calumet Area Water Quality Committee in the late 1960's under the auspices of the Federal Water Pollution Control Administration (FWPCA). A series of meetings and symposia were initiated to assess the effectiveness of on-going pollution control programs and the relative progress toward meeting water quality objectives. The Technical Committee on Water Quality (1970) concluded that no significant improvement in water quality had been realized over the 1965 baseline condition despite discharger compliance with then-existing water quality criteria and requirements. With the replacement of the FWPCA by the U.S. Environmental Protection Agency (USEPA), a nationwide intiative was instituted. Emphasis shifted from the site specific implementation of the Calumet Area Water Quality Committee to a broad based USEPA water pollution control program, which incorporated many of the previous recommendations.

The basin's poor water quality and potential effects on the adjacent nearshore area of Lake Michigan prompted the drafting of a master plan for the Grand Calumet River (U.S. Environmental Protection Agency, 1985) to identify or develop required remedial actions, additional pollution control needs, and an implementation schedule and plan. The USEPA has identified a series of objectives and goals within the context of the master plan to improve water quality within the Grand Calumet River. Through the National Pollution Discharge Elimination System (NPDES) permit program, more stringent effluent limitations may be

TABLE 2. Chemical and physical properties of nineteen point source dischargers in the Grand Calumet River Basin during July and August 1986.

Outfall	pH	Hardness (mg/L $CaCO^3$)	Alkalinity (mg/L $CaCO^3$)	Specific Conductance (μS)
East Chicago	7.78	247-259	149-160	1464
East Chicago[a]	8.10	133-138	253-265	1575
Gary STP	8.10	227-255	149-159	728
Hammond STP	7.90	200-256	235-249	1119
U.S. X				
002	8.22	115-147	112-154	279
007	8.32	128-142	112-126	278
010	7.81	137-145	112-124	378
018	8.04	117-143	100-106	252
020	7.39	125-142	100-120	282
030	8.29	132-147	113-126	330
034	7.80	211-224	98-109	535
E.I. DuPont de Nemours				
003	7.42	510-540	30-40	8670
003[a]	7.22	392-400	24-30	8480
LTV Steel				
009	8.05	133-150	101-109	355
010	7.98	154-175	110-124	434
011	8.39	116-139	60-64	441
Inland Steel Co				
002	8.17	90-147	96-117	286
008	8.10	104-138	104-119	301
008[a]	8.24	101-108	138-145	339
011	8.66	117-156	105-129	300
012	8.30	131-142	103-114	329
014	8.26	151-189	88-103	458
014[a]	7.87	148-164	88-96	467

[a] Water chemistry for definitive sample.

instituted to minimize the introduction of toxic pollutants currently entering the river. USEPA is focusing attention on improving the immediate river channel. The current study assesses the sub-chronic toxicity of 19 major point source dischargers along the Grand Calumet River and Indiana Harbor Canal, using the embryo-larval survival and teratogenicity test.

MATERIALS AND METHODS

All tests of industrial and municipal effluents were conducted under similar conditions by personnel of the USEPA, Central Regional Laboratory in Chicago,

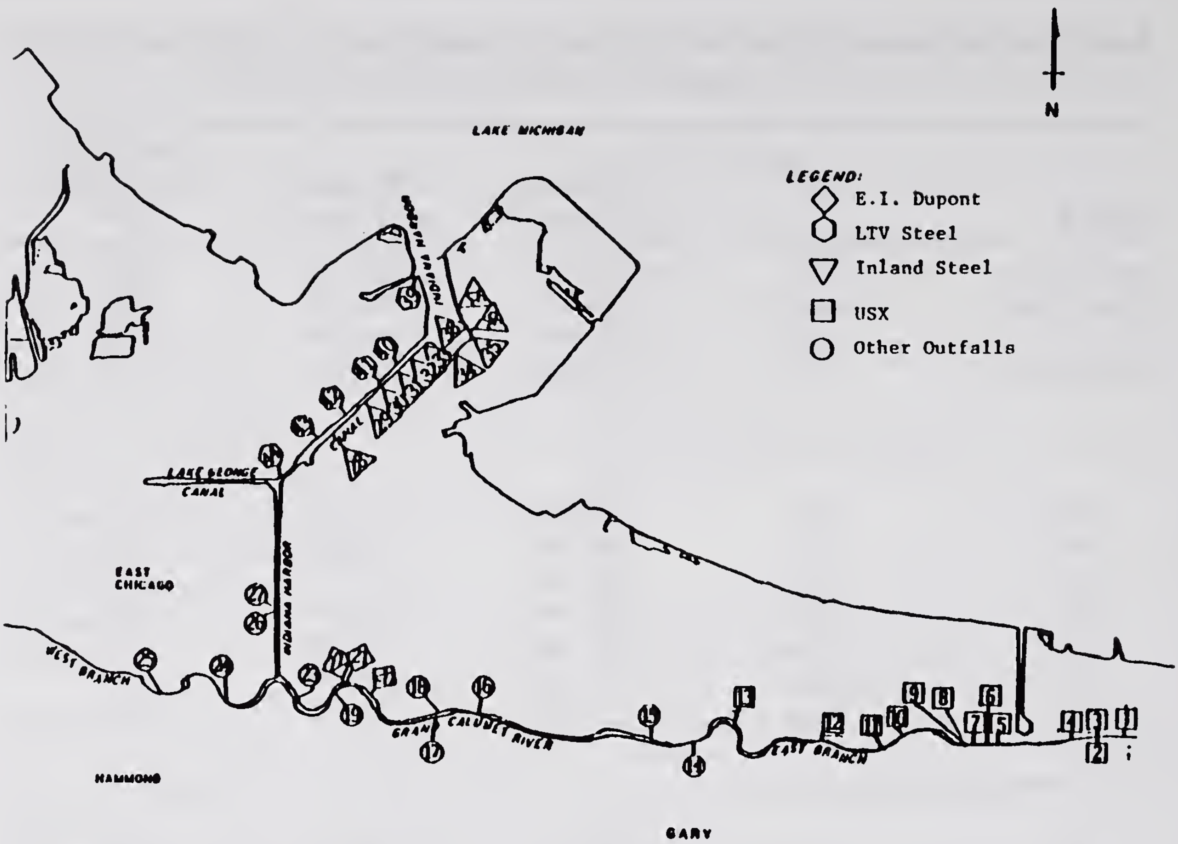

FIGURE 1. Point source dischargers in the Grand Calumet River basin, Indiana (Modified from ISBH, 1984).

Illinois. The tests were conducted within 24 hrs of sample collection as static, 8-day, daily renewal tests in a walk-in environmental chamber capable of regulating temperature, humidity, and photoperiod. Water temperatures were sustained within ± 1° C of 24° C. Photoperiod was set at 16 hrs light and 8 hrs dark. Standard culture methods and techniques were employed by personnel of the USEPA, Environmental Support Laboratory, Biological Methods Branch, Aquatic Biology Section, Newtown, Cincinnati, Ohio, following methods outlined in Klemm (1985). Fathead minnow *(Pimephales promelas)* eggs from the prior 24 hrs spawn were collected from spawning tiles at Newtown and shipped express mail in styrofoam containers to Chicago. Eggs were all spawned over an 8 hr period and were less than 48 hrs old at the initiation of each test.

The specific methods of the test follow Horning and Weber (1985) and were adapted from Birge and Black (1981) and Birge, *et al.* (1985) with a few modifications documented in *Standard Operating Procedures for Conducting Chronic, Static Embryo-Larval Survival and Teratogenicity Testing of Effluents* (U.S. Environmental Protection Agency, 1986). A summary of the test methods is presented in Table 1. Modifications to the test procedure include: lowering the volume of test solution to 400 ml; use of an egg cup with nitex mesh screening to facilitate egg and larvae manipulation during the test; sample collected as a single grab sample refrigerated at 4° C for the duration of the eight days; and deletion of teratogen-affected larvae (which were alive at the conclusion of the test) from the dead category for the derivation of the chronic value.

The deletion of abnormal fathead minnow larvae from the calculation of the chronic value removed any subjective bias on the part of the analyst in distin-

TABLE 3. Preliminary test results for municipal and industrial dischargers in the Grand Calumet River Basin during July and August 1986.

Effluent Concentration	Percent Hatchability	Relative Percent Teratogenicity Alive	Dead	Total	Percent Survival A	B	x̄
Control[a]	97	0	0	0	94	88	91
Hammond STP (100%)	97	0	7.2	7.2	80	96	88
Gary STP	95	0	1.0	1.0	84	94	89
East Chicago	99	7.1	26.3	33.3	2	46	24*
DuPont de Nemours							
003	100	—	—	—	0	0	0*
USX							
002	99	0	0	0	100	92	96
007	97	0	0	0	92	86	89
010	97	56.7	0	56.7*	0	0	0*
018	91	0	0	0	86	98	92
020	98	0	0	0	94	—	94
030	98	1.0	0	1.0	90	84	87
034	80*	0	0	0	7	6	6.5*
Control[b]	100	0	1	1	100	98	99
LTV Steel							
009	98	0	0	0	98	88	93
010	99	0	0	0	88	96	92
011	94	1.1	0	1.1	94	82	88
Inland Steel							
022	100	2.0	0	2.0	92	92	92
008	100	0	0	0	88	88	88*
011	96	0	0	0	90	92	91
012	100	0	2.0	2.0	90	94	92
014	99	0	2.0	2.0	88	72	80*

* Signifies a significant deviation between the control and test concentration using Students t-test ($P \leq 0.05$)

[a] Control sample for Hammond STP, Gary STP, East Chicago SD, DuPont de Nemours, and USX outfalls.

[b] Control sample for LTV Steel and Inland Steel outfalls.

guishing the severity of teratogenicity. The expression of teratogenicity is dependent on the age of the egg, since differences in exposure periods (e.g., exposure during cleavage) may significantly enhance or reduce the teratogenic response. To ensure that all eggs were affected by the effluent and did not die due to lack of fertilization, only embryos ranging between 48 to 72 hrs old at the initiation of the test were used. This enabled the analyst to distinguish between fertile and nonfertile eggs prior to placement into the test dilution series. A decreasing di-

TABLE 4. Definitive test results of municipal dischargers exhibiting preliminary screen chronic toxicity, teratogenicity, or hatchability deviations from the control.

Effluent Concentration	Percent Hatchability	Relative Percent Teratogenicity Alive	Dead	Total	Percent Survival A	B	x̄
East Chicago STP							
0	96	0	0	0	96	98	97
3	98	4.2	1.0	5.2*	84	96	90
10	98	2.0	1.0	1.3*	98	90	94
30	98	0	1.0	1.0*	100	92	96
60	98	1.0	3.0	4.0*	84	100	92
100	94	7.4	30.9	38.3*	92	68	80

* Signifies a significant deviation from the control using Student t-test.

lution series comprised of five effluent concentrations (3%, 10%, 30%, 60%, and 100%) was used for definitive testing. Control and dilution water was charcoal-filtered Lake Michigan water from the USEPA, Central Regional Laboratory—Fish Culture Unit, which was filtered through a 0.22 μm filter to remove any deleterious microorganisms.

During the course of preliminary screening, effluent at three test sites was routinely monitored to ensure sample integrity. The following parameters were monitored: ammonia, hardness, alkalinity, pH, and specific conductivity. No significant deviation was observed over the eight-day period of any parameter during the testing period for East Chicago Sanitary District, Gary Public Owned Treatment Works, and USX outfall 010. The decision was made that a single sample could be utilized in the daily renewal process for the duration of the test.

The endpoints evaluated in the study were the following: survival, hatchability, and relative percent teratogenicity. Routine monitoring of the physical properties of the test solution and their control on a daily basis included: temperature, dissolved oxygen, specific conductance, pH, alkalinity, and hardness (Table 2). Since many tests were conducted over a short time span, all tests were initially conducted as preliminary screens with retesting of outfalls which showed statistically significant differences from the control using Dunnett's procedure.

Statistical analysis. Mortality data from the study was analyzed by Dunnett's Procedure after transforming the square root of the proportion of dead organisms to an arc sine value. Dunnett's Procedure consists of an analysis of variance (ANOVA) to determine the error term, which is then used for comparing each of the treatment means with the control mean in a series of paired tests. The data was checked to ensure that observations were independent and normally distributed with homogeneity of variance. Normality was tested using a chi-square goodness of fit test, and homogeneity of variance was verified using Bartlett's Test (Horning and Weber, 1985; Snedecor and Cochran, 1980). Hatchability and relative percent teratogenicity were compared using a binomial distribution and a one-tailed t-test ($P \leqslant 0.05$).

During definitive testing, the chronic value was calculated using no observed effect concentration (NOEC) and the lowest observed effect concentration. The chronic value was derived by calculating the geometric mean of the highest concentration that caused no effect and the lowest statistically significant ($P \leq 0.05$) effect concentration for survival. Dunnett's procedure was used to evaluate definitive results, while Student's t-test was used to determined differences in hatchability, teratogenic response, and preliminary test survival.

Hydrology. The Grand Calumet basin is located in the northwestern corner of Indiana and adjacent areas of Illinois (Figure 1). The basin encompasses approximately 43,242 acres and is almost entirely contained within Lake County, Indiana (U.S. Environmental Protection Agency, 1982). The Grand Calumet River is bordered by the Little Calumet River to the south and Lake Michigan to the north. The east branch of the river originates at Marquet Park Lagoon, east of Gary, Indiana. From the headwaters, the river flows approximately 13 miles to the west, where it is joined by the river's west branch and empties into the Indiana Harbor Canal, approximately 3 miles east of the Illinois State Line. The Indiana Harbor Canal then flows north/northeast for approximately 5 miles and empties into southern Lake Michigan.

Flow within the system is sluggish and is estimated to average 16 cfs in the west branch and 880 cfs in the east branch (U.S. Environmental Protection Agency, 1982). Flow is sustained by high discharged volumes of cooling water derived from Lake Michigan, which is pumped to the river from USX. More then 90% of the flow originates as treated municipal and industrial wastewater, industrial cooling and process water, and stormwater runoff (U.S. Environmental Protection Agency, 1982). Frequent flow reversals are known to occur, extending into the west branch of the Grand Calumet River. The extent of flow reversals is dependent on the stage of Lake Michigan.

RESULTS

Public owned treatment works. Three publicly owned treatment works (POTW) representing the cities of Hammond, Gary, and East Chicago discharge into the Grand Calumet River. Prior to discharge, the Hammond Sanitary District (SD) uses a chlorine contact baffle system, while East Chicago SD and Gary POTW have holding ponds with final clarifiers.

During the preliminary screens, no significant sub-chronic mortality was observed in either the Hammond Sanitary District or Gary POTW effluents (Table 3). A teratogenic response was observed in 1.0% of the Gary POTW exposed larvae. East Chicago SD had a statistically significant deviation in survival from the control (t-values = 6.04). Significant teratogenic effects were observed in both 100% effluent concentrations of East Chicago SD and Hammond SD, with 33.3% and 7.2% of the larvae exhibiting a response, respectively. Hammond SD larvae possessed edematous yolk sacs and deformed mandibles, clear blood, and tumors on the nape and anterior trunk. Responses observed in East Chicago SD larvae included deformed crania and yolk sacs, absence of mandibles, body tumors, post-caudal body swellings, clear blood, and reduced heart beat.

The East Chicago SD was retested with fresh samples to determine dilution effects. No significant deviation in mortality (t-test; $P \geq 0.05$) was observed between any dilution and the control. Significant teratogenic responses were observed at all dilutions except 30% (Table 4). No detrimental effect was observed

TABLE 5. Industrial discharger definitive chronic embryo-larval testing results from the Grand Calumet River Basin during July and August 1986.

Effluent Concentration	Percent Hatchability	Relative Percent Teratogenicity			Survival		
		Alive	Dead	Total	A	B	x̄
Inland Steel 008							
0	100	0	0	0	90	98	94
3	99	0	1.0	1.0	98	94	96
10	94	0	2.1	2.1	88	78	83
30	100	0	0	0	100	96	98
60	96	0	2.0	2.0	96	86	91
100	84*	1	0	1.1	70	62	66*
Inland Steel 014							
0	100	0	0	0	90	98	94
3	97	0	0	0	94	98	96
10	100	1.0	5.5	6*	98	100	99
30	98	0	4.1	4.1	100	94	97
60	95	2.1	7.4	9.5*	56	80	68*
100	74*	5.4	2.7	8.1	2	0	1*
DuPont de Nemours							
0	96	0	0	0	96	98	97
3	95	0	2.1	2.1	96	86	90
10	98	0	2.0	2.0	100	96	98
30	98	35.7	30.6	66.3*	66	20	43*
60	98	65.3	1.0	66.3*	0	2	1*
100	97	65.9	0	65.9	0	0	0*

* Signifies a significant deviation from the control.

in hatchability among the three treatment facilities during either preliminary or definitive testing.

Industrial dischargers. Major dischargers in the Grand Calumet River—Indiana Harbor Canal include USX (previously U.S. Steel—Gary Works), LTV Steel, and Inland Steel. Only outfalls, which discharged contact, process, or treated water, were sampled. USX has 20 permitted outfalls discharging into the east branch of the Grand Calumet River, seven of which satisfied the above-stated criteria. LTV and Inland Steel discharge through 9 and 13 permitted outfalls, respectively, into Indiana Harbor and the Indiana Harbor Canal. Five outfalls were tested at LTV and Inland Steel. However, LTV Steel outfall 001 was non-operational, and outfall 008 has been permanently closed since 1979.

E.I. DuPont de Nemours and Company manufactures inorganic industrial chemicals, including herbicides and fungicides. DuPont discharges into the east branch of the Grand Calumet River through 3 outfalls and also utilizes the East Chicago SD. A single outfall (003) was sampled from E.I. DuPont.

Five of the seven USX outfalls did not elicite a sub-chronic mortality response. However, outfall 010 caused complete mortality of all test organisms, and 034 had limited survivorship (Table 3). Teratogenic responses were observed only in outfalls 010 and 030. Only the response at outfall 030 was statistically significant (t-test; $P \geqslant 0.05$) with 56.7% of the hatched larvae exhibiting a response. Teratogenic responses included clear blood, reduced heart beat, deformed mandibles, edematous yolk sacs, and swellings on the body. A significant difference in hatchability was observed only at outfall 034 (80% hatch). A USX strike prevented any additional definitive testing from being conducted during 1986.

LTV steel effluents did not exhibit any reduction in survival, hatchability, or teratogenic response during preliminary testing (Table 3). As a result, no additional testing was deemed necessary.

Preliminary testing of 3 of 5 Inland Steel outfalls did not elicite a sub-chronic response (Table 3). A statistically significant difference in survivorship was observed at outfall 008. Although greater mortality was observed at outfall 014, the result was not significant ($P \geqslant 0.05$) in a one-tailed Student t-test. The increased variance between the replicates was probably caused by a Type II error in accepting a false null hypothesis. A nonsignificant teratogenic response was observed at outfalls 002, 012, and 014.

Definitive sub-chronic testing of Inland Steel outfalls 008 and 014 was conducted with fresh effluent. A statistically significant difference in survivorship was observed in 100% effluent from outfall 008 and at concentrations above 60% for outfall 014 (Table 5). Outfall 008 had a NOEC of 60% effluent and a LOEC of 100% effluent. A chronic value of 77.5% effluent with a minimum significant difference (MSD) of 0.099 represented a 10.52% reduction in survival. A teratogenic response was exhibited in dilutions as low as 3% effluent at outfall 008 and was significant in dilutions as low as 10% effluent for outfall 014. Statistically significant responses in hatchability were found for 100% effluent at outfall 008 and 014.

E.I. DuPont de Nemours and Company outfall 003 caused complete mortality of newly hatched larvae after 120 hrs of exposure in the preliminary test (Table 3). DuPont effluent had no effect on the embryonic developmental stage, since no reduction in hatchability was observed. However, teratogenic response was not adequately assessed, since the larvae did not survive longer than 24 hrs after hatching. Definitive testing of fresh DuPont effluent confirmed the preliminary testing results (Table 5). Within 168 hrs of exposure, significant mortality was observed in concentrations above 30% effluent. A chronic value of 17.3% effluent was calculated from a NOEC of 10% and LOEC of 30% effluent. The MSD of 0.184 corresponded to a 18.92% reduction in survival. Hatchability during definitive testing was unaffected. However, teratogenic responses were observed in all concentrations. Statistically significant differences in teratogenic response was observed in dilutions above 30% effluent.

DISCUSSION

The general contaminated nature of the river sediments and overlying water column has been documented in the Grand Calumet River basin (U.S. Department of the Interior, 1966, 1967; U.S. Environmental Protection Agency, Technical Committee on Water Quality, 1970, 1982, 1985). Toxicants are known to be discharged from permitted point source outfalls into the river. Recent improvements in treatment technologies and effluent control have resulted in reductions of point

TABLE 6. Summarized chemistry data for Grand Calumet River Basin definitive embryo-larval chronic toxicity testing.

Effluent/ Compound		East Chicago		Dupont 003		Inland Steel 008		Inland Steel 014	
		Total	Dissolved	Total	Dissolved	Total	Dissolved	Total	Dissolved
Metals	Ag µg/L	<6	<6	<6	<6	<6	<6	<6	<6
	Al	186	<80	<80	<80	<80	<80	<80	<80
	B	490	487	81	83	84	84	<80	115
	Ba	36	28	<6	30	19	19	<6	24
	Be	<1	<1	<1	<1	<1	<1	<1	<1
	Cd	—	—	—	—	<10	<10	<10	<
	Co	<6	<6	<6	<6	<6	<6	<6	<6
	Cr	<8	<8	<8	<8	<8	<8	<8	<8
	Cu	11	10	<6	7	7	7	21	21
	Fe	685	123	106	88	<80	<80	<80	2800
	Li	24	26	<10	40	<10	<10	<10	20
	Mn	118	112	<5	8	5	5	<5	65
	Mo	<15	<15	<15	<15	<15	<15	<15	<15
	Ni	<15	<15	<15	<15	<15	<15	<15	<15
	Sn	<40	<40	<40	<40	<40	<40	<40	<40
	Sn	<40	<40	<40	<40	<40	<40	<40	<40
	Sr	208	213*	<10	194*	116	116*	<10	147*
	Ti	<25	<25	<25	<25	<25	<25	<25	<25
	V	<5	<5	<5	<5	<5	<5	<5	<5
	Y	<5	<5	<5	<5	<5	<5	<5	<5
	Zn	81	<40	<40	58*	<40	<40	<40	141*
	Ca mg/L	72	74	<.5	158	35	35	<.5	45
	K	7	7	<.1	2	10	10	<.1	11
	Mg	17	17	<.1	2	10	10	<.1	11
	Na	221	218*	1	1700*	6	6	<1	14
	As µg/L	5	3	<20	<20	<2	<2	<2	<2
	Se	16	16*	<2	<2	<2	<2	<2	<2
	Cd	<1	<1	<1	<1	.2	.3*	.3	.6*
	Pb	17	11*	<2	<2	4	10*	81	25*
	Hg	0.2	0.3*	0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Hexavalent Chromium µg Cr/L		<10		<10		<10		<10	
Total Organic Carbon mg C/L		10		<3		<3		6	
Ammonia mg N/L		0.35*		<0.5		<0.5		<0.5	
Phenols µg Phenolics/L		30		26		28		40	
Total Cyanide µg Cn/L		300*		<5		<5		19	

Oil and Grease mg/L	<5	<5	<5	7
GC/MS semi -volatile				
Organic scan for priority pollutants	NS[a]	NS	NS	NS
VOA	NS	NS	NS	NS

[a] Data was either below detection or was not significant.
* Signifies that element or compound may be a toxic agent.

source loading rates. However, a substantial reservoir of these materials is contained in river sediments.

The control of toxic levels of compounds entering the river can be achieved by monitoring these outfalls. Chemical-specific criteria are a very expensive and intensive way of regulating toxic loadings and do not take into consideration the inhibitory, additive, or synergistic combinations possible in the receiving stream.

In the present study, analysis of water samples was initiated only on samples during definitive testing. None of the samples had detectable levels of volatiles, semivolatiles, oil and grease, or phenolics. Suspected contaminants identified included strontium, sodium, copper, zinc, selenium, cyanide, ammonia, mercury, and lead (Table 6).

Cyanide levels of 13.3 μg/L to 20.2 μg/L have been observed to cause chronic toxicity within fathead minnows (Lind, *et al.,* 1977). Cyanide levels of 300 μg/L were observed in East Chicago SD, and 19 μg/L in Inland Steel outfall 014. High cyanide levels apparently did not cause the toxic effect in East Chicago, since no significant mortality was observed. Cyanide may be a teratogen, since a response was observed in 33.3% of the preliminary screen larvae and in a mean of 66.5% of the larvae in definitive tests between 30% and 100% effluent.

Ammonia occurred at significant levels in East Chicago SD effluent and at Inland Steel outfall 014. Thurston, *et al.* (1986) found fathead minnow spawning affected at chronic ammonia levels ranging from 0.8 mg/L to 0.9 mg/L and reduced hatching success at 0.19 mg/L and higher. East Chicago SD ammonia levels were 0.35 mg/L, while Inland Steel outfall 014 levels were 0.49 mg/L.

Rainbow trout chronically exposed to lead concentrations of 27 μg/L during their early life stages developed spinal deformities in 32.2% of the fish (Davies, *et al.,* 1976). Total and dissolved lead levels were in close agreement for East Chicago SD and Inland Steel outfall 008 but were in disagreement at Inland Steel outfall 014 (Table 5). The dissolved level of lead at Inland Steel outfall 014 represented only 30.9% of total lead. Lead effects are inversely proportional to water hardness, with greatest toxicity observed at low hardness concentrations. Hardness levels at East Chicago SD and Inland Steel were about 5 to 7 times greater than the test conditions reported by Davies, *et al.* (1976). Sauter, *et al.* (1977) reported that lead amounts of 71 μg/L to 146 μg/L correspond to chronic toxicity effects in rainbow trout at a hardness value of 35 mg/L.

Zinc was observed to produce a chronic toxicity effect in laboratory testing in both hard water (Brungs, 1969) and soft water (Benoit and Holcombe, 1978), suggesting that zinc chronic toxicity may be relatively unaffected by hardness. Benoit and Holcombe (1978) reported chronic effects for fathead minnows using zinc in the range of 78 μg/L to 145 μg/L. Inland Steel outfall 014 had total zinc values of 141 μg/L. However, agreement between total and dissoved zinc (which was 3.5 times greater) did not exist. Zinc concentrations at outfall 014 were great enough to be of interest synergistically but not as an individual contaiminant.

Lind, *et al.* (cited in Stephan and Gentile, 1980) reported chronic effects to fathead minnows from copper in the range of 13.1 μg/L to 26.6 μg/L. The effect of copper on fathead minnows was not hardness dependent (Mount, 1968; Mount and Stephen, 1969; Pickering, *et al.*, 1977). A decrease in chronic toxicity with increasing hardness is suspected for most other species (U.S. Environmental Protection Agency, 1985). Total copper levels were 6.79 μg/L and 21.4 μg/L at Inland Steel outfalls 008 and 014, respectively. A dissoved copper value of 8.3 μg/L was found at outfall 008, while about half of the total copper occurred at outfall 014.

Call, *et al.* (1983) and Snarski and Olson (1982) reported mercury levels below 0.26 μg/L to have chronic toxicity impacts on fathead minnows. Effluent from East Chicago SD had dissolved mercury levels of 0.3 μg/L.

Salinity is known to enhance the chronic toxicity responses to otherwise nonharmful contaminents such as phenolics (European Inland Fisheries Advisory Commission, 1973). High sodium levels, with close agreement between total and dissolved sodium, at E.I. DuPont de Nemours outfall 003 may have enhanced the synergistic contribution of other contaminants.

The sublethal teratogenic effects observed in this study suggested that a synergistic combination produced the abnormalities at DuPont outfall 003. Copper and lead may have jointly contributed to observed teratogenicity at Inland Steel outfall 008 and 014. High levels of cyanide in East Chicago SD did not cause a reduction in survival but in conjunction with high ammonia levels may have caused significant teratogenicity. Thurston, *et al.* (1986) exposed fathead minnows from larval to adult stages to varying concentrations of ammonia. Swollen darkened areas on the heads occurred at concentrations of 0.21 mg/L NH_3 and higher, while observable growths, which often displaced the eyes, were visible at concentrations of 0.42 mg/L NH_3 and higher. Thurston, *et al.* (1986) reported a positive correlation between the occurrence of lesions and increased ammonia concentration. Gillespie and Baumann (1986) collected bluegill adults previously exposed to high levels of selenium and artifically cross-fertilized their gametes. Larvae exposed to high levels of selenium while undergoing embryogenesis exhibited edematous yolk sacs within 100 hr after hatching. These larvae were also less motile and died prior to reaching swim-up stage. Gillespie and Bauman (1986) suggested that their observations were due to transformation from the female to the eggs during oogenesis. Levels less than 30 μg/L did not cause significant mortality to carp, zebra danio, and rainbow trout during embryonic or larval stages (Huckabee and Griffith, 1974; Niimi and Latham, 1975; Goettl and Davies, 1978; Hodson, *et al.*, 1980). Gillespie and Baumann (1986) reported 100% edematous bluegill larvae in lakes which had an average of 10 μg/L selenium, a figure well below the water quality criterion for selenium of 35 μg/L.

Of equal importance in evaluating ambient teratogenicity is the finding of Birchfield (1987), who reported cranial anomalies from field collected specimens of gizzard shad and bluegill due to capture technique, fixation, or handling.

Hatching success was significantly inhibited only in 100% effluent from Inland Steel outfalls 008 and 014. Although neither copper or lead were at significant levels, the synergistic combination of these metals may have affected hatching.

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