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PREDICTIVE ABILITIES OF ENVIRONMENTAL PROTECTION AGENCY SUBCHRONIC TOXICITY TEST ENDPOINTS FOR COMPLEX EFFLUENTS

Thomas P. Simon, Arthur Lubin, and Frank Le Curieux
U.S. Environmental Protection Agency, Region V
Central Regional Laboratory
Chicago, Illinois 60605

ABSTRACT: Seven endpoints from three EPA short-term chronic toxicity tests were evaluated for their abilities to predict impacts from various complex effluents. Compared were the subchronic fathead minnow embryo-larval survival and teratogenicity test, fathead minnow larval growth and survival test, and *Ceriodaphnia* reproduction and survival tests for ten Standard Industrial Classification (SIC) codes from 75 Region V point source dischargers. Statistical methods were used to compare correlation coefficients (r) for endpoints within and between tests, determine patterns among process types, and establish predictions of endpoints suitable for each of the major process types. Separate sub-chronic endpoint measurements should be calculated in order to determine the greatest amount of information per test. Successful response levels were highest for larval survival, embryo-larval survival, and *Ceriodaphnia* reproduction endpoints, and lowest for larval growth. Correlation coefficients suggest that a high degree of redundancy exists among the *Ceriodaphnia* survival and reproduction endpoints, and to a lesser extent embryo-larval hatching and survival, and larval growth and *Ceriodaphnia* survival. Differences in statistical significance of correlation coefficients between various SIC codes, suggest that endpoint relationships are not the same among the SIC code categories.

INTRODUCTION

Research and development of methods for quantifying chronic toxicity has rapidly progressed during the 1980s. Previously, chronic toxicity was measured using extensive effort in performing full scale life-cycle tests. The Environmental Protection Agency (EPA) has developed short-term sub-chronic tests (USEPA, 1989) to replace the full-scale methods. Test methodology utilizes fathead minnow larvae in a growth and survival test (Norberg and Mount, 1983), and embryos in a survival and teratogenicity test (Birge *et al.*, 1985). *Ceriodaphnia dubia* neonates are tested to evaluate trends in survival and reproduction including the number of broods and young (Mount and Norberg, 1984). The simplification of the subchronic tests and the reduction in time has greatly increased the utility of the methods by concentrating on the most sensitive life stage during the species early life history. The proliferation of laboratories conducting toxicity tests has been a direct result of EPA's inclusion of these tests as biological monitoring requirements in industrial and municipal permits.

The need for a rapid screening procedure for increasing a laboratories ability to process samples has produced the current battery of tests for water quality management decisions. The ability to *a priori* evaluate whether a particular test might provide a result for a discharger's effluent would be a valuable management tool. Determination of data redundancy, endpoint overlap, and sensitivity of tests reduce the work load to a level where more tests could be conducted.

Table 1. Standard Industrial Classification (SIC) codes for ten Region V industrial and municipal process types (USEPA 1984).

Code	Process Type	Acronym
1000	Ore Mining Dressing	ORM
2400	Timber Product Processing	TPP
2600	Pulp and Paperboard Mills and Converted Paper Products	PPMCPP
2700	Paint and Ink Formulating and Printing	PIFP
2800	Inorganic Chemical Manufacturing	ICM
3000	Rubber Processing	RP
3300	Iron and Steel Manufacturing	ISM
3400	Home Machinery and Mechanical Product Manufacturing	HMMPM
3700	Automotive Parts and Accessories	APA
4900	Sewerage Systems	SS

The current study evaluates test results from 75 Region V point source dischargers distributed over 10 Standard Industrial Classification (SIC) codes. The objectives were to evaluate each of the seven sub-chronic test endpoints, based on each SIC code, to determine the degree of redundant data; the patterns among SIC code categories; and the *a priori* predictability of results based on SIC code for the chronic toxicity discharger data set. Although the data set presented is a preliminary evaluation of trends, it is representative of Region V and includes all comparative testing completed by the USEPA Region V, Central Regional Laboratory. Information presented is a preliminary account, additional research is warranted to develop trends for each of the specific SIC codes.

METHODS AND MATERIALS

The EPA categorized dischargers into a series of process types to aid in information retrieval for the evaluation of point source discharger compliance through the National Pollutant Discharge Elimination System (NPDES) permit program (USEPA, 1984). Ten process types were evaluated for the current study and included a finer breakdown within certain process types to determine facility patterns (Table 1). Acronyms were developed for each of the ten process types to maintain brevity, the SIC code will follow the acronym for convenience in parentheses.

Chronic toxicity was quantified from 75 industrial and municipal facilities in Region V, and included five Canadian effluents which discharge into the Great Lakes. Samples were composited over a 24 hr period or were collected as grab samples. In each case the tests were conducted using the same methodology. Samples were either mailed or hand delivered to the Central Regional Laboratory, Chicago by field personnel in order for tests to be initiated within 36 hr after sample collection. Timing of the sample began at the end of sample collection. Field water collection methods and laboratory test procedures are based on the established national EPA method protocols (USEPA, 1985).

Testing conducted included the fathead minnow embryo-larval survival and teratogenicity test, larval survival and growth test, and *Ceriodaphnia* survival and reproduction test (Table 2). Data results for each of the seven endpoints were weighted according to test results for the embryo-larval survival and teratogenicity endpoints (e.g. if 90 eggs hatched in the embryo-larval test, with 75 larvae surviving until the conclusion

Table 2. Sub-chronic toxicity tests, endpoints, test duration and test organisms conducted by the US Environmental Protection Agency, Region V.

Test	Chronic Endpoint	Test Duration	Organism
Embryo-Larval	Hatching Teratogenicity Survival	8 days	*Pimephales promelas*
Larval Growth	Survival Growth	7 days	*Pimephales promelas*
Ceriodaphnia Reproduction and Survival	Survival Reproduction	7 days	*Ceriodaphnia dubia*

of the test, the final survival of fathead minnow larvae would be scored as 83.3% rather than 75%). If greater than 20% mortality affected the upper dilutions, no attempt was made to calculate endpoints for *Ceriodaphnia* reproduction or larval growth endpoints in 100% effluent.

Several departures from data interpretation protocol were made from the EPA chronic manual in order to further evaluate effects from the effluent. Teratogenic larvae were not considered dead unless they expired either during or at the conclusion of the test. Teratogenicity was a separate endpoint based on gross signs of external deformation or erratic behavior. Gross signs of external deformation included edematous yolk sacs, cranial or caudal swellings, tumors, deformed eyes or mandible, lack of blood pigment, non-fusiform shape, kyphosis, lordoscoliosis, rigid coiling of the vertebral column, scioliosis, and cephalic or symmetric twinning. Aberrant behavior observations include reduced heart beat, inability to swim, inability to feed, and whirling (Simon, 1988).

Correlation analysis was used to evaluate similarity among the endpoints. Correlation is a measure of the degree that variables vary together or a measure of the strength of associations. All statistical analyses were computed using the Statistical Analysis System (SAS) software package (Blalock, 1972; SAS, 1982, 1985; Steel and Torrie, 1960).

Outliers, which may impact correlation results, were not addressed in the current study since standard toxicants and within concentration consistency provided quality assured results and were shown to be accurate. The data set compared, although small, is the largest comparative national data set for the three tests using common test solutions. Descriptive statistics are provided for each endpoint in order to evaluate the magnitude and degree of toxicity exhibited for the 75 effluents from the current study (Table 3).

RESULTS AND DISCUSSION

Correlation Analysis

Correlation analysis for all SIC codes categories (between code comparison) indicated that a high degree of correlation existed only for the *Ceriodaphnia* survival and reproduction endpoints ($r = 0.96223$). Possible strong correlations may also be apparent for embryo-larval hatch and survival endpoints ($r = 0.85045$). Between SIC code comparisons for all of the data indicated that no further endpoints were significantly correlated (Table 4).

Table 3. Actual toxicity observed in a cumulative data set acquired from 75 point source dischargers in Region V. Mean toxicity, standard deviation, t-value and statistical significance for seven sub-chronic endpoints used by the U.S. Environmental Protection Agency Region V, for evaluating chronic toxicity of complex effluents. N equals the number of tests conducted.

Endpoint	N	Mean	Standard Deviation	T-statistic
Embryo-Larval Hatching	67	76.41	36.79	5.29[a]
Embryo-Larval Teratogenicity	67	76.41	39.80	5.26[a]
Embryo-Larval Survival	67	71.13	35.43	6.67[a]
Larval Survival	24	59.99	38.72	5.06[a]
Larval Growth	24	91.78	27.28	1.48
Ceriodaphnia Survival	45	71.01	38.29	5.08[a]
Ceriodaphnia Reproduction	42	64.44	39.26	5.87[a]

[a] Statistical significance at a probability level of < 0.01.

Table 4. Correlation coefficients for a cumulative data set of ten Standard Industrial Classification (SIC) codes.

	Embryo-Larval Hatching (1)	Embryo-Larval Teratogenicity (2)	Embryo-Larval Survival (3)	Larval Survival (4)	Larval Growth (5)	Ceriodaphnia Survival (6)	Ceriodaphnia Reproduction (7)
1	—						
2	0.30017	—					
3	0.85045*	0.39965	—				
4	0.61116	0.49717	0.26403	—			
5	0.43229	−0.27569	0.34139	0.28480	—		
6	0.08403	0.11835	0.14110	0.65318	0.74152*	—	
7	0.04509	0.03764	0.09358	0.43691	0.50484	0.96223*	—

*Statistically significant at $p \leq 0.05$.

Table 5. Correlation coefficients for Standard Industrial Classification (SIC) codes from the U.S. Environmental Protection Agency, Region V. a). 2600, b). 2800, c). 3300, and d). 4900.

	Embryo-Larval Hatching (1)	Embryo-Larval Teratogenicity (2)	Embryo-Larval Survival (3)	Larval Survival (4)	Larval Growth (5)	Ceriodaphnia Survival (6)	Ceriodaphnia Reproduction (7)
1	—						
2	−0.03261	—					
3	0.39970	−0.17597	—				
4	1.00000	−0.33333	—	—			
5	—	—	—	—	—		
6	0.10825	0.02684	0.54098	—	—	—	
7	0.13501	0.07243	0.56799	0.42379	—	0.99514	—
A. Pulp and Paperboard Mills and Converted Paper Products (PPMCPP)							
1	—						
2	0.53936	—					
3	0.93254	0.52387	—				
4	0.82535	−0.44424	−0.27657	—			
5	0.57735	−0.92325	0.63166	0.43541	—		
6	0.63143	0.18103	0.63387	0.77393	0.76608	—	
7	0.22843	−0.03116	0.37072	0.45693	0.59493	0.21686	—
B. Inorganic Chemical Manufacturing (ICM)							
1	—						
2	0.18111	—					
3	0.25304	0.78097	—				
4	—	—	—	—			
5	—	—	—	—	—	—	
6	−0.26055	−0.20000	—	—	—	—	
7	−0.21954	0.25790	—	—	—	0.89507	—
C.Iron and Steel Manufacturing (ISM)							
1	—						
2	0.48084	—					
3	0.73764	0.61143	—				
4	0.71004	0.80513	0.52040	—			
5	0.40499	−0.29277	0.08462	0.07718	—		
6	0.47164	0.70919	0.73802	0.68058	0.71963	—	
7	0.56344	0.22832	0.04804	0.40203	0.50445	0.57964	—
D. Sewerage System (SS)							

Table 6. Patterns of positive responses (percent expression of toxicity from total) for seven subchronic toxicity endpoints for ten Standard Industrial Classification (SIC) code categories within U.S. Environmental Protection Agency, Region V.

	Embryo-Larval				Larval-Growth			Ceriodaphnia		
SIC Code	N	% Hatch	% Terata	% Survival	N	% Survival	% Growth	N	% Survival	% Reproduction
1000	1	0	0	0	0	—	—	1	100.0	100.0
2400	1	100.0	0	100.0	1	100.0	100.0	1	0	100.0
2600	9	66.7	44.4	44.4	5	20.0	0	8	0	50.0
2700	0	—	—	—	1	0	0	1	0	0
2800	10	50.0	70.0	100.0	5	80.0	0	9	66.7	40.0
3000	1	0	0	100.0	1	100.0	0	1	100.0	100.0
3300	23	21.7	13.0	30.4	2	50.0	0	6	16.7	33.3
3400	1	0	100.0	100.0	0	—	—	0	—	—
3700	1	100.0	100.0	100.0	0	—	—	2	100.0	100.0
4900	20	27.8	40.0	50.0	9	75.0	11.1	16	56.3	58.3
TOTAL	67	35.8	35.8	52.2	24	60.9	12.5	45	44.4	52.4 [a]

[a] Total number of endpoints were based on 42 tests.

When evaluating patterns within SIC codes (Table 5), a high degree of correlation for embryo-larval hatching and larval survival for PPMCPP (r = 1.000), ICM (r = 0.82535), and SS (r = 0.71004). *Ceriodaphnia* reproduction and survival endpoints were highly correlated for PPMCPP, SIC codes 2600 (r = 0.99514), and ISM, SIC 3300 (r = 0.89507), but not for ICM, SIC codes 2800 (r = 0.21686) or SS, SIC code 4900 (r = 0.57964). Embryo-larval hatching and survival were highly correlated for ICM, SIC codes 2800 (r = 0.93254) and SS, code 4900 (r = 0.73764) but not for PPMCPP, SIC codes 2600 (r = 0.39970) or ISM, SIC codes 3300 (r = 0.25304). Embryo-larval hatching was also correlated with larval survival for PPMCPP (r = 1.000), ICM (r = 0.82535), and SS (r = 0.71004).

Fathead minnow embryo-larval survival and larval survival endpoints were not highly correlated for any category comparison. The highest degree of correlation was for sewerage systems SIC code 4900 (r = 0.52040). This is not surprising since the larval stage of the fish being compared, *Pimephales promelas* in the embryo-larval and larval growth tests is a 4 and 7 day posthatch larva, respectively. The 4 day posthatching larva possesses a yolksac and is not actively feeding, instead subsisting on endogenous yolk platelets, while the later has absorbed its yolk and is subsisting on exogenous food sources. Larval growth and *Ceriodaphnia* survival were correlated for ICM, SIC codes 2800 (r = 0.76608) and SS, SIC code 4900 (r = 0.71963). The degree of consistency was in response to the tendency towards no observed response for either of these test endpoints. Based on the differences between SIC code categories observed in the correlation analysis, none of the seven endpoints should be combined as is recommended in the national guidance.

Patterns in Toxicity Response

The patterns of toxicity expressed by dischargers within each of the 10 SIC code categories were evaluated by looking at the frequency of positive response for each of the endpoints (Table 6). A positive response was defined as any chronic toxicity value which could be calculated. The chronic value is based on the geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC).

Test endpoints exhibiting the greatest sensitivity included the larval survival, embryo-larval survival, and *Ceriodaphnia* reproduction. Larval survival had the highest response frequency exhibiting an effect in 60.9% of the tests conducted. *Ceriodaphnia* reproduction and embryo-larval survival responded in approximately half of the exposures, and embryo-larval hatching, embryo-larval teratogenicity, and *Ceriodaphnia* survival responded in a third of the tests conducted. The larval growth endpoint was the least responsive with an observed effect in only 12.5% of the exposures. Test responses were statistically significant (t-test; $p \leq 0.01$), for all endpoints except for the larval growth endpoint (Table 3). This suggests that certain endpoints may be better evaluators of specific industrial and municipal effluents. Reduction in the number of tests per effluent would enable more effluents to be tested.

Within SIC code comparisons, the embryo-larval survival endpoint was responsive all of the time for process type TPP (SIC code 2400), ICM (2800), RP (3000), HMMPM (3400), and APA (3700). The endpoint was responsive half of the time for process types PPMCPP (2600) and SS (4900) and a third of the time for exposures in category ISM (3300). The larval survival endpoint was responsive all of the time for process types TPP (2400) and RP (3000), and in at least 75% of the exposures from process types ICM (2800), RP (3000), and SS (4900).

Comparisons between embryo-larval and larval survival of *P. promelas* indicates very little correlation exists. In cases where both tests were conducted on the same effluent, similar results were observed for TPP (2400) and RP (3000). Embryo-larval survival endpoint performed best (t-test; $p > 0.05$), e.g. was more sensitive, than larval survival in process types PPMCPP (2600) and ICM (2800). The inverse was true for only process type SS (code 4900).

Due to the low number of tests conducted in most test categories, data may be applicable only for SIC code categories PPMCPP (2600), ICM (2800), ISM (3300), and SS (4900). Further data interpretation may be necessary for the additional categories.

Predictability

Even though the current data base includes major point source dischargers representative for Region V, the predictability of endpoint outcomes from toxicity tests will be difficult for the majority of SIC codes. The patterns observed from the frequency distribution and correlation analysis supports that the embryo-larval survival and teratogenicity test should be conducted with effluents from process types PPMCPP (2600), ICM (2800) and SS (4900). The larval survival and growth test should be conducted on process types TPP (2400), ICM (2800), RP (3000), ISM (3300) and SS (4900). The *Ceriodaphnia* survival and reproduction test provides the most sensitive endpoint response for the majority of process types including ORM (1000), TPP (2400), PPMCPP (2600), ICM (2800), RP (3000), APA (3700), and SS (4900)(Table 6).

Statistical significance (t-test; $p \leq 0.01$), was observed for six of the seven endpoints when the cumulative data set was studied (Table 3). The exception was the larval growth endpoint which was not statistically significant ($\alpha > 0.05$).

Due to the lack of observations for some SIC code categories, further statistical analysis was possible only for SIC codes process types PPMCPP (2600), ICM (2800), ISM (3300), and SS (4900)(Table 7). Only the embryo-larval hatching endpoint was

Table 7. T-values for four Standard Industrial Classification (SIC) code categories for determining statistical significance of actual chronic toxicity response.

	SIC Code Categories			
Endpoints	2600	2800	3300	4900
Embryo-Larval Hatch	3.33[a]	3.56[b]	1.76[a]	2.29[a]
Embryo-Larval Teratogenicity	2.10[b]	4.33[b]	1.28	3.02[b]
Embryo-Larval Survival	1.38	7.80[b]	2.51[b]	3.74[b]
Larval Survival	0.92	3.64[a]	0[b]	3.73
Larval Growth	0	0.95	0	0.91
Ceriodaphnia				
	0	3.33[b]	0.91	3.69[b]
Ceriodaphnia Reproduction	1.91[b]	2.31[a]	1.35	3.52[b]

[a] Statistically significant at $p < 0.05$;
[b] Statistically significant at $p < 0.01$.

statistically significant (t-test; $p \leq 0.05$) across the four SIC code categories. The larval survival endpoint, although statistically significant in the cumulative data set, was not significant (t-test; $p > 0.05$) for SIC code categories ICM (2800) and ISM (3300). The greatest amount of statistical significance was observed in SIC code categories ICM (2800) and SS (4900), with only the larval growth endpoint being non-significant. Within SIC code category 2600, four of the seven endpoints were not statistically significant (t-test; $p < 0.05$) including embryo-larval and larval survival, larval growth, and *Ceriodaphnia* survival. The least amount of statistical significance was observed in process type ISM (3300) where five of the seven endpoints were below the $p < 0.05$ level. The larval growth endpoint was not statistically significant for any of the four SIC code categories. Based on the correlation analysis and t-test comparisons, the utility of a multiple, non-redundant set of endpoints for the subchronic toxicity testing being conducted by EPA is supported.

The current study was the first attempt to correlate test response with industrial and municipal process types. No other critical analysis of the subchronic toxicity test battery has been attempted and include the tests in the current study. Further comparative testing within other media, e.g. leachate, groundwater, and ambient toxicity samples including water and sediment, should likewise be critically reviewed in order to evaluate response patterns.

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