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RELATION OF SURVIVAL TO OTHER ENDPOINTS IN CHRONIC TOXICITY TESTS WITH FISH

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Abstract—Hazard assessments of chemicals in aquatic organisms often include chronic toxicity testing. The evaluation of exposure duration and of the life stages tested according to standard test methods has led to the development of shorter chronic toxicity tests. A similar evaluation of biological endpoints (i.e., survival, growth and reproduction) could result in tests that are more economical. We analyzed endpoints for 28 chemicals and seven fish species in 34 chronic toxicity studies. When all endpoints were compared, survival was equal to or more sensitive than all other endpoints 56 to 69% of the time. Individual endpoints were more sensitive than survival 19 to 61% of the time, except for reproduction, which was always more sensitive (although there were few observations). The no observed effect concentration (NOEC) for growth could be predicted from the NOEC for survival by using interendpoint correlations ($r = 0.949$ to 0.974). Ratios of NOECs for survival to those for all other endpoints examined were 5 or less in 93 to 96% of the comparisons (specific endpoint comparisons ranged from 80 to 100%).

The determination of the survival endpoint requires less time and money than does the determination of most other endpoints, and it appears adequate for hazard assessments in the initial stage of estimating chronic toxicity. However, a factor of at least 0.2 should be applied to the estimated no-effect concentrations for survival to include other potential biologically significant effects at least 95% of the time. The factor of 0.2 is based on frequency analyses that resulted in the NOECs for survival being 5 times or less than the NOECs for most other endpoints about 95% of the time. Univariate analyses, however, indicated a range of 0.13 to 0.22 for the factor. A thorough evaluation of other published studies that contain endpoints other then survival should be conducted to define the appropriate factor more accurately.

Keywords—Chronic toxicity Endpoints Survival Fish Hazard assessment

INTRODUCTION

Chronic toxicity tests commonly include the measurement of long-term effects of a contaminant on the survival, growth and reproduction of a test organism. Such studies generally are expensive, high-risk investigations requiring 6 months to a year to conduct. Consequently, there is much interest in developing alternative methods that can provide similar information with less effort and expense. Macek and Sleight [1] and McKim [2] reported that 30- to 90-d exposures of embryo-larval and early juvenile life stages are sufficient for estimating the maximum acceptable toxicant concentration as described by Mount and Stephan [3]. Furthermore, Ward and Parrish [4] reported that the survival endpoint is as sensitive as growth endpoints, or more so. Mayer et al. [5] and Mehrle and Mayer [6] proposed the use of biochemical and physiological endpoints to shorten partial and full life-cycle chronic toxicity tests, since survival, growth and reproduction are the culmination of many biochemical phenomena that occur in regulated patterns. Because biochemical changes caused by a toxicant should occur before reductions in survival, growth or reproduction are observed, appropriate biochemical tests might be developed to estimate chronic toxicity and decrease

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Table 1. Chronic no-effect concentrations (μg/L) of various chemicals for fry survival, growth, reproduction and gross pathology for seven species of fish

Chemical and fish species[a]	Days of exposure	Survival	Growth		Reproduction	Gross pathology; other	Source
			Weight	Length			
Aroclor 1254							
Brook trout	48	9.0	1.0				Mauch et al. [20]
	118	9.0	9.0	>13			
Aroclor 1254/1260							
Rainbow trout	90	2.1	>2.9	2.1		>2.9[b,c]	Mayer et al. [21]
Arsenic pentoxide							
Rainbow trout	30	>2,900	>2,900	1,940			Unpublished data
	60	>2,900	>2,900	1,140			
	90	>2,900	>2,900	1,140			
Arsenic trioxide							
Coho salmon	140	>300	>300	>300			Nichols et al. [22]
	175	>300	>300	>300		17–170[d]	
Chlordecone							
Fathead minnows	30	1.9	>5.1	>5.1			Buckler et al. [23], Mehrle et al. [24]
	60	1.9	>5.1	1.9			
Fathead minnows[e]	120	>0.31	>0.31	>0.31	0.23[f]	0.11[g]	
Cumylphenyl diphenyl phosphate							
Rainbow trout	30	0.56		>2.0			Mayer et al. [25]
	60	1.7	>2.0	1.0			
	90	1.7	>2.0	1.0		1.0[h]	
2,4-D butyl ester							
Lake trout	60	44	22	22			Woodward and Mayer [26]
2,4-D propylene glycol butyl ether ester							
Lake trout	60	72	72	72			Woodward and Mayer [26]
DEF (tributylphosphorotrithioate)							
Rainbow trout	30	3.1	3.1	3.1			Cleveland and Hamilton [27]
	90			4.8			
Channel catfish	30	6.9	>20	6.9			
	60	14	3.1	3.1			
	90	14	3.1	3.1			
Fyrquel GT							
Fathead minnows	30	134	310	310			Unpublished data
	60	134	310	310			
	90	134	310	310		>500[h]	
Kronitex							
Fathead minnows	30	>88	>88	>88			Unpublished data
	60	>88	>88	>88			
	90	>88	>88	>88		>88[h]	

Methoxychlor							
Rainbow trout	30	4.6		1.9			Unpublished data
	60	1.9		1.9			
	90	1.9	1.9	1.9			
Mirex							
Fathead minnows[e]	120	>34	>34	>34	9.5[i]	>34[g]	Buckler et al. [23], Mehrle et. al. [24]
Monoethanolamine							
Brook trout	30	14,100		14,100			Unpublished data
	60	14,100	14,100	14,100			
	90	14,100	14,100	14,100			
Brook trout[e]	100	>20,000	>20,000	>20,000	1,770[f,j]		
Nonylphenyl diphenyl phosphate							
Rainbow trout	30	>4.2		3.0			Mayer et al. [25]
	60	>4.2	>4.2	0.88			
	90	>4.2	>4.2	1.8		1.8[h]	
Pentachlorophenol, Dowicide EC-7							
Fathead minnows	30	>140	>140	>140			Cleveland et al. [28]
	60	>140	>140	>140			
	90	>140	>140	>140		>140[b]	
Pentachlorophenol, industrial composite							
Fathead minnows	30	42	8.8	8.8			Cleveland et al. [28]
	60	19	8.8	8.8			
	90	19	8.8	8.8		19[b]	
Pentachlorophenol, purified							
Fathead minnows	30	>140	>140	55			Cleveland et al. [28]
	60	>140	55	55			
	90	>140	55	55		>140[b]	
Petroleum, crude[k]							
Cutthroat trout	30	480		120			Woodward et al. [29]
	60	480	<100	≤100			
	90	480	<100	≤100		120–260[b,h,j]	
Petroleum, refined[k]							
Cutthroat trout	30	130		30			Woodward et al. [30]
	60	130	30	30			
	90	130	30	30		47[b,m]	
Phosflex 31P							
Fathead minnows	30	77	77	77			Unpublished data
	60	77	>200	77			
	90	77	77	77		>200[h]	

(*continued*)

Table 1 (continued)

Chemical and fish species[a]	Days of exposure	Growth		Reproduction	Gross pathology; other	Source
		Survival	Weight	Length		
Pydraul 50E						
Rainbow trout	90	11	5.6	3.0	1.6[h]	Mayer et al. [25], unpublished data
Lake trout	90	9.2	3.7	3.7		
	120	9.2	9.2	3.7	3.7[h]	
Fathead minnows	30	490	490	490		Mayer et al. [25]
	60	490	>750	490		
	90	490	>750	490	210[h]	
Pydraul 115E						
Rainbow trout	30	>34		10		Mayer et al. [25]
	60	>34		<5.8		
	90	10	<5.8	<5.8	<5.8[h]	
Sodium selenite						
Rainbow trout	30	31		68		Unpublished data
	60	31		31		
	90	31	68	68		
TFM (3-trifluoromethyl-4-nitrophenol)						
Brook trout	30	2,530		2,530		Dwyer et al. [31]
	60	1,230		1,230		
	90	1,230		1,230		
Brook trout[c]	120	5,930		5,930	2,530[j]	11,900[h]
Toxpahene						
Brook trout	30	0.051		0.097		Mayer et al. [32]
	60	0.097		<0.039		
	90	0.097	0.097	0.097		
Brook trout[c]	90	>0.50	0.38	>0.50		
	180	0.20	0.20	>0.50	0.051[j]	
Fathead minnows	30	>0.17	0.13	0.13		Mayer et al. [33]
Fathead minnows[c]	98	>0.17	0.072	0.072	>0.17[f]	
Channel catfish	30	0.20	0.20	0.096		Mayer et al. [33]
	60	0.096	0.20	0.20		
	90	0.096	0.20	0.20		
Triphenyl phosphate						
Rainbow trout	90	>1.4	>1.4	>1.4	>1.4[h]	Mayer et al. [25]
Fathead minnows	30	140		>230	>230[h]	Mayer et al. [25]

Transformer oil							
Rainbow trout	30	100		50		50[b]	Mayer et al. [21]
	60	100		100		50[b]	
	90	400	50	100		100–400[b,n]	

[a]Scientific names of fish: coho salmon, *Oncorhynchus nerka*; cutthroat trout, *Salmo clarki*; rainbow trout, *S. gairdneri*; brook trout, *Salvelinus fontinalis*; lake trout, *S. namaycush*; fathead minnow, *Pimephales promelas*; channel catfish, *Ictalurus punctatus*.
[b]Fin erosion.
[c]Hematocrit, serum cortisol, serum protein, swimbladder volume and disease susceptibility, >2.9 μg/L.
[d]Plasma thyroxine, 17 μg/L; gill ATPase, 55 μg/L; chloride cells in gills and smoltification, 170 μg/L.
[e]Adults.
[f]Percent hatch.
[g]Spinal curvature.
[h]Cataract in one or both eyes.
[i]Egg production.
[j]Percent egg viability.
[k]Water-soluble fraction.
[l]Gill lesions, 260 μg/L.
[m]Gill and liver lesions and swimming performance, 47 μg/L.
[n]Hematocrit, 100 μg/L; serum cortisol, 200 μg/L; serum protein, >570 μg/L; swimbladder volume, 200 μg/L; disease susceptibility, 400 μg/L.

the exposure time now required. However, in a review of the literature, Tucker and Leitzke [7] found that, generally, no sublethal effect now measurable occurs at a concentration of less than one-sixth that producing a comparable percent mortality in equivalent tests.

The Toxic Substances Control Act of 1976 (PL 94-469) and the Federal Insecticide, Fungicide, and Rodenticide Act (PL 80-104), as amended by the Federal Environmental Pesticide Control Act of 1972 and others (7 U.S.C. 136-136y), authorize the U.S. Environmental Protection Agency to obtain data from industry on the health and environmental effects of chemical substances and mixtures. It has been reported that there is insufficient information available for a complete hazard assessment of any of the 50,000 chemicals now in commercial use that are not pesticides, cosmetics, drugs or food additives [8]. This situation is complicated by the introduction of about 1,000 new chemicals each year [9]. The lack of testing capabilities for the timely screening of such a large array of chemicals may require the use of shortened approaches. We therefore evaluated various endpoints to determine the extent to which no-effect concentrations of one endpoint, such as survival, could be used to predict the no-effect concentrations of other endpoints. Our results may allow the use of fewer, more economical tests for the initial evaluation of environmentally hazardous chemicals.

MATERIALS AND METHODS

All 34 studies using 28 chemicals and seven fish species were conducted under similar conditions by personnel of the Columbia National Fisheries Research Laboratory, with the exception of one study conducted at the Seattle National Fisheries Research Center with coho salmon (*Oncorhynchus kisutch*) and arsenic trioxide. The tests were conducted in flow-through diluter systems modeled after that described by Mount and Brungs [10]. Each diluter delivered five to seven concentrations of toxicant and a control. Water temperature was maintained within ±1°C of the desired temperature, and day length was regulated by the method of Drummond and Dawson [11]. Eggs and fish were cultured in accordance with the procedures of Brauhn and Schoettger [12]. The specific methods for experimental design and endpoint measurements are included among the published articles cited in Tables 1 through 3. Concentrations of all chemicals except monoethanolamine were measured.

The endpoints evaluated in the study were the

Table 2. Chronic no-effect concentrations (μg/L) of various chemicals for biochemical constituents of vertebrae of fry[a]

Chemical and fish species	Days of exposure	Organic				Inorganic		Inorganic/ organic ratio
		Collagen	Hydroxyproline	Proline	Proline/ hydroxyproline ratio	Calcium	Phosphorus	
Aroclor 1254								
Brook trout	48		1.0					
	118	>9.0	>9.0			0.54	<0.43	
Aroclor 1254/1260								
Rainbow trout	90	>2.9	>2.9	>2.9	>2.9	>2.9	>2.9	>2.9
Arsenic pentoxide								
Rainbow trout	90	>2,900	>2,900	>2,900	>2,900	>2,900	>2,900	>2,900
Chlordecone								
Fathead minnows	30		0.11					
Fathead minnows[b]	120	0.11	>0.31			>0.31	>0.31	>0.31
Cumylphenyl diphenyl phosphate								
Rainbow trout	30		>2.0	>2.0	>2.0	>2.0	>2.0	
	60		>2.0	>2.0	>2.0	<0.22	>2.0	
	90	>2.0	>2.0	>2.0	1.2	>2.0	>2.0	>2.0
DEF (tributylphosphorotrithioate)								
Rainbow trout	90	>9.5	>9.5	>9.5	>9.5	>9.5	>9.5	>9.5
Channel catfish	90	>20	>20	>20	6.9	>20	3.1	>20
Fyrquel GT								
Fathead minnows	90	>500	>500	>500	>500	>500	>500	>500
Kronitex 200								
Fathead minnows	90	>88	>88	>88	>88	9.5	>88	>88
Methoxychlor								
Rainbow trout	90	0.36	>1.9					
Mirex								
Fathead minnows[b]	120	>34	>34			>34	>34	>34
Nonylphenyl diphenyl phosphate								
Rainbow trout	30		>4.2	>4.2	>4.2	>4.2	3.0	
	60		>4.2	>4.2	0.52	>4.2	>4.2	
	90	>4.2	0.52	>4.2	1.1	2.6	>4.2	>4.2
Phosflex 31P								
Fathead minnows	90	>77	>77	>77	>77	>77	<8.0	>77
Pydraul 50E								
Rainbow trout	90	0.95	>6.2	>6.2	>6.2	>6.2	>6.2	>6.2
Fathead minnows	90	490	>750	210	>750	>750	>750	>750

Pydraul 115E										
Rainbow trout	30		>34	>34	>34	>34	>34			
	60		>34	>34	>34	<5.8	<5.8			
	90	>34	23	>34	12	>34	>34	>34		
Sodium selenite										
Rainbow trout	90	>68	>68	>68	>68	10	>68	>68		
Toxaphene										
Brook trout	30		0.097			>0.29	>0.29			
	60		<0.039			>0.14	0.051			
	90		<0.039			<0.039	<0.039			
Fathead minnows	30		0.072							
Fathead minnows[b]	98	0.072	>0.17			>0.17	>0.17			
Channel catfish	90	0.059	0.059			<0.049	0.096			
Triphenyl phosphate										
Rainbow trout	90	>1.4	0.63	1.1	>1.4	>1.4	>1.4	>1.4		
Transformer oil										
Rainbow trout	90	200	>570	50	400	200	200	400		

[a] See Table 1 for data sources.
[b] Adults.

following: survival; growth—length and weight; reproduction—egg production, viability and hatchability; histopathology—gross pathology (cataracts, fin erosion, spinal curvature) and tissue lesions of gill, kidney, liver and swimbladder; clinical characteristics—blood (cortisol, hematocrit, leucocrit, protein, thyroxine), disease susceptibility, gill ATPase, vertebrae (biochemical [seven characteristics], density, mechanical [five characteristics]); and, behavior—smoltification and swimming performance.

Statistical analysis

Because the raw data in all studies were reanalyzed statistically, the significance reported here may vary from that given in previous publications. A repeated measurement (split plot) analysis of variance was performed to determine the effect of different concentrations over time. The linear statistical model proposed by Gill and Hafs [13] included the main effects of concentration and time, and the interaction of concentration and time. Replication within concentrations was used as an error to test the main effect of concentration. When the experimental unit was not measured repeatedly, a two-way factorial design was used [13]; the linear statistical model then included the main effects of concentration and time, and the interaction of concentration and time. If only one time period was present, a simple one-way analysis of variance was calculated using concentration as the effect. Treatment means were compared with control means by the least-significant-difference test [14]. A row × column chi-square test [15] had to be used with some data arranged in two classes because replication was inadequate.

The no observed effect concentration (NOEC) 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 each endpoint. Although significant differences were sometimes noted at lower concentrations, only the lowest of the consistently significant concentrations was used. Ratios of NOECs for survival to those for other endpoints were derived, and frequency analyses were conducted on all ratios, first with greater-than values categorized by numerical ratio and then with greater-than values deleted. Both data sets were analyzed to determine if the use of only real numbers (greater-than values deleted) would alter the frequency distributions. Univariate analyses [16] were also applied to the data.

Table 3. Chronic no-effect concentrations (μg/L) of various chemicals for density and mechanical properties of vertebrae of fry[a]

Chemical and fish species	Days of exposure	Density	Strength		Elasticity		Toughness
			Rupture	Elastic limit	Strain	Modulus of elasticity	
Aroclor 1254/1260							
Rainbow trout	90	>2.9	>2.9	>2.9	>2.9	>2.9	>2.9
Arsenic pentoxide							
Rainbow trout	90	2,200	>2,900	>2,900	750	>2,900	>2,900
DEF (tributylphosphorotrithioate)							
Rainbow trout	90	1.1	>9.5	>9.5	>9.5	>9.5	>9.5
Channel catfish	90	3.1	3.1	3.1	>20	3.1	3.1
Sodium selenite							
Rainbow trout	90	>47	>47	>47	>47	>47	31
Transformer oil							
Rainbow trout	90	200	400	400	>570	400	400

[a]See Table 1 for data sources.

Regression analyses (interendpoint correlations), using Model II least-squares methodology [17], were conducted to determine relationships between NOECs for survival and growth (weight or length) among all species and chemicals in various time periods. Slopes and intercepts were derived from the relationship $\log y = a + b(\log x)$, where y is the NOEC for survival and x is the NOEC (ng/L) for weight or length.

RESULTS AND DISCUSSION

The NOEC was used for endpoint comparisons instead of the 50% effect concentration used by Tucker and Leitzke [7] because results with aquatic organisms in many studies have not followed a graded dose-response curve and effects reflect more of an "all or none" response. The ratios of the NOECs for survival to those for each of the other endpoints for a particular chemical were evaluated using all ratios, and with greater-than ratios deleted (Table 4). No substantial differences were evident in frequency distributions between the two data sets. Comparison of NOECs for 28 chemicals showed that survival was a very sensitive indicator of chronic toxicity, even though the comparisons were not always balanced (not all endpoints were represented in all studies).

Overall, the sensitivity of survival exceeded that of all other endpoints combined 41 to 51% of the time and equaled or exceeded that of the other endpoints 56 to 69% of the time. Individual endpoints were more sensitive than survival 19 to 61% of the time, except for reproduction, which was always more sensitive (although there were few observations). Comparisons were also made with survival and gross pathology combined (lowest NOEC of the two endpoints used to calculate ratios), since severe fin erosion, spinal curvature and cataracts could impair survival. The ratios exceeded 1.0 in 20 to 32% of the comparisons but were 5.0 or less 86 to 92% of the time. Combining length with survival and gross pathology increased the occurrence of ratios in the 1.0 or less category, indicating that the sensitivity of one of the three endpoints would be equal to or greater than that of all other endpoints 86 to 90% of the time.

Univariate analyses [16] provided more clearly defined ratios below which 25, 50, 75, 90, 95 or 99% of the observations occurred (Table 5). The comparison of survival versus all other endpoints resulted in 95% of the ratios falling below 4.6 to 7.5. In other words, the NOEC for survival could be multiplied by a factor of 0.13 to 0.22 (reciprocals of 7.5 and 4.6) to include the NOEC for all other endpoints 95% of the time. By frequency analyses (Table 4), a ratio of 5.0 or less included all values 95% (93 to 96%) of the time, which would result in a factor of 0.20. The ratios required to include 99% of the values were considerably higher.

The NOECs for growth (length and weight) were more sensitive than those for survival 25 to 37% of the time (Table 4); the percentage was not substantially increased by including reproduction and gross pathology (40 to 54%). However, 83 to

Table 4. Frequencies of survival to other endpoint ratios

Endpoint Comparisons	Cumulative frequencies in ratios of:							
	<1.0	1.0	1.1–2.0	2.1–3.0	3.1–4.0	4.1–5.0	5.1–10.0	10.1–20.0
Survival vs. all other endpoints								
% (*n*)[a]	41 (126)	56 (171)	70 (212)	82 (248)	86 (260)	93 (283)	98 (298)	100 (304)[b]
% (*n*)[c]	51 (126)	69 (171)	79 (196)	86 (212)	89 (220)	96 (238)	99 (244)	100 (247)
Survival or gross pathology vs. all other endpoints[d]								
% (*n*)[a]	53 (160)	68 (205)	78 (236)	86 (261)	88 (268)	95 (287)	98 (297)	100 (303)[b]
% (*n*)[c]	62 (160)	80 (205)	87 (223)	90 (231)	92 (235)	98 (251)	99 (254)	100 (256)
Survival vs. weight, length, reproduction and gross pathology								
% (*n*)[a]	23 (34)	46 (69)	61 (91)	77 (116)	83 (124)	94 (141)	97 (145)	100 (146)
% (*n*)[c]	30 (34)	60 (69)	71 (82)	82 (94)	87 (100)	97 (112)	100 (115)	
Survival, length or gross pathology vs. all other endpoints[d]								
% (*n*)[a]	70 (186)	86 (227)	90 (239)	94 (247)	95 (252)	97 (255)	98 (260)	100 (264)[b]
% (*n*)[c]	74 (186)	90 (227)	94 (236)	96 (240)	97 (244)	98 (246)	99 (249)	100 (251)
Survival vs weight								
% (*n*)[a]	30 (15)	54 (27)	68 (34)	80 (40)	80 (40)	96 (48)	100 (50)	
% (*n*)[c]	36 (15)	64 (27)	74 (31)	81 (34)	81 (34)	95 (40)	100 (42)	
Survival vs length								
% (*n*)[a]	19 (14)	48 (35)	63 (46)	81 (59)	86 (63)	99 (72)	100 (73)	
% (*n*)[c]	25 (14)	62 (35)	75 (42)	84 (47)	89 (50)	100 (56)		
Survival vs. reproduction								
% (*n*)[a]			20 (1)	40 (2)	80 (4)	80 (4)	80 (4)	100 (5)
% (*n*)[c]				50 (1)	100 (2)			
Survival vs. gross pathology								
% (*n*)[a]	28 (5)	39 (7)	56 (10)	83 (15)	94 (17)	94 (17)	100 (18)	
% (*n*)[c]	33 (5)	47 (7)	60 (9)	80 (12)	93 (14)	93 (14)	100 (15)	
Survival vs. biochemical endpoints of vertebrae								
% (*n*)[a]	63 (73)	67 (77)	77 (89)	85 (98)	87 (100)	88 (101)	96 (111)	100 (115)[b]
% (*n*)[c]	77 (73)	81 (77)	89 (85)	92 (87)	93 (88)	94 (89)	97 (92)	100 (95)
Survival vs. mechanical endpoints of vertebrae								
% (*n*)[a]	50 (13)	69 (18)	77 (20)	77 (20)	81 (21)	100 (26)		
% (*n*)[c]	54 (13)	75 (18)	79 (19)	79 (19)	79 (19)	100 (24)		
Survival vs. uncategorized endpoints[e]								
% (*n*)[a]	35 (6)	41 (7)	70 (12)	82 (14)	88 (15)	88 (15)	94 (16)	100 (17)
% (*n*)[c]	46 (6)	54 (7)	77 (10)	92 (12)	100 (13)			

[a]Includes all values; greater-than (>) values categorized by numerical ratio.
[b]One biochemical endpoint of vertebrae was >21.
[c]Greater-than values deleted.
[d]Lowest NOEC used as numerator.
[e]See footnotes c, d and l through n to Table 1.

87% of the ratios of survival to all four endpoints combined and compared together were 5.0 or less. These results basically agree with those of Ward and Parrish [4] for sheepshead minows (*Cyprinodon variegatus*), in which effect concentrations for survival were equal to or less than effect concentrations for growth in 17 of 18 tests (94%). In a more recent study, Woltering [18] examined 173 tests and found, as we did, that fry survival and growth were often equally sensitive. Growth was

Table 5. Univariate analyses of survival to other endpoint ratios

Endpoint Comparisons	*n*	Mean	Mode	Range	Quantiles (ratios below which *x*% of the observations occur) 25%	50%	75%	90%	95%	99%
Survival vs. all other endpoints										
All values[a]	304	2.1	1.0	0.10–21	0.69	1.0	2.5	4.5	7.5	17
Real values[b]	247	1.6	1.0	0.10–17	0.61	1.0	2.0	4.3	4.6	15
Survival or gross pathology vs. all other endpoints[c]										
All values[a]	303	1.7	1.0	0.13–21	0.43	1.0	1.9	4.5	5.4	17
Real values[b]	256	1.2	1.0	0.13–17	0.43	0.72	1.0	2.7	4.5	12
Survival vs. weight, length, reproduction and gross pathology										
All values[a]	146	2.0	1.0	0.27–11	1.0	1.4	2.5	4.5	4.8	10
Real values[b]	115	1.8	1.0	0.27–9.0	0.65	1.0	2.2	4.3	4.6	8.8
Survival, length or gross pathology vs. all other endpoints										
All values[a]	264	1.2	1.0	0.13–21	0.37	0.60	1.0	2.0	3.9	17
Real values[b]	251	0.93	1.0	0.13–17	0.35	0.50	1.0	1.0	2.3	13
Survival vs. weight										
All values[a]	50	2.0	1.0	0.34–9.0	0.70	1.0	2.5	4.8	6.2	9.0
Real values[b]	42	1.9	1.0	0.34–9.0	0.61	1.0	2.2	4.6	7.5	9.0
Survival vs. length										
All values[a]	73	1.9	1.0	0.28–5.6	1.0	1.4	2.5	4.4	4.8	5.6
Real values[b]	56	1.6	1.0	0.28–4.8	0.77	1.0	2.1	4.3	4.5	4.8
Survival vs. reproduction										
All values[a]	5	4.4	1.3	1.3–11	1.8	3.6	7.4	11	11	11
Survival vs. gross pathology										
All values[a]	18	2.1	1.0	0.27–6.9	0.69	1.8	2.8	4.3	6.9	6.9
Real values[b]	15	2.0	1.0	0.27–6.9	0.61	1.7	2.8	5.2	6.9	6.9
Survival vs biochemical endpoints of vertebrae										
All values[a]	115	2.1	1.0	0.10–21	0.46	0.85	2.0	6.6	9.4	20
Real values[b]	95	1.5	1.0	0.10–17	0.33	0.72	1.0	2.5	8.2	17
Survival vs. mechanical endpoints of vertebrae										
All values[a]	26	1.7	0.72	0.66–4.5	0.70	0.86	2.5	4.5	4.5	4.5
Real values[b]	24	1.6	0.72	0.66–4.5	0.70	0.72	1.8	4.5	4.5	4.5
Survival vs. uncategorized endpoints[d]										
All values[a]	17	2.8	0.72	0.70–18	0.72	1.8	2.8	7.8	18	18
Real values[b]	13	1.6	0.72	0.70–4.0	0.72	1.0	2.4	3.5	4.0	4.0

[a]Greater-than (>) values categorized by numerical ratio.
[b]Greater-than values deleted.
[c]Lowest NOEC used as numerator.
[d]See footnotes c, d and l through n to Table 1.

not of critical importance in establishing NOEC values in 86% of the tests, and, in the tests where growth was the single most sensitive endpoint (14%), fry survival could be used to estimate the NOEC within an average factor of 3.

Growth effects were predictable from survival effects (Table 6). Length was less variable than weight, and although all of the correlation coefficients (r) exceeded 0.9, they were slightly higher for length (0.970 to 0.974) than for weight (0.949 to 0.965). Also, no alteration was noted in the intercepts for length versus survival between 30 and 90 d of exposure; the intercepts of weight versus survival varied, without trends, over time.

Table 6. Interendpoint correlations[a] of survival and growth

Analysis and days of exposure	n	Intercept (a)	Slope[b] (b)	Correlation coefficient (r)	$\bar{y} \pm 95\%$ C.I.
Weight vs. survival					
30	6	0.395	0.920	0.965	4.35 ± 0.41
60	10	0.682	0.901	0.949	4.63 ± 0.31
90	15	0.194	0.993	0.957	4.33 ± 0.22
Length vs. survival					
30	16	0.284	0.968	0.972	4.64 ± 0.18
60	17	0.263	0.965	0.974	4.60 ± 0.15
90	18	0.275	0.971	0.970	4.41 ± 0.17

[a]$\log y = a + b(\log x)$, where y is no-effect concentration for survival and x is no-effect concentration (ng/L) for growth (length or weight).
[b]All slopes were significantly different from 0 ($p \leq 0.01$).

These observations further support the hypotheses of others [1,2,4] that 30-d exposures of embryo-larval and early juvenile life stages are sufficient for estimating NOECs.

After reviewing several studies, Tucker and Leitzke [7] concluded that no more than a sixfold difference in median effect concentrations can be produced using any known biochemical, histopathological or behavioral effect as the endpoint in place of lethality. Of 304 ratios in the present evaluation, only 21 (6.9%) were 5.1 or higher, and most of these were biochemical characteristics of fish vertebrae or clinical characteristics associated with salmon smoltification. Although statistical significance was observed with some biochemical endpoints in fish vertebrae, it did not necessarily mean that bone quality (in terms of mechanical properties) was decreased. When sodium selenite and transformer oil were tested against rainbow trout (*Salmo gairdneri*), biochemical changes occurred at concentrations of one-eighth to one-fifth those causing significant changes in vertebral strength or elasticity. However, concentrations causing changes in biochemical and mechanical properties of vertebrae were about the same when arsenic pentoxide and DEF were tested against rainbow trout and channel catfish (*Ictalurus punctatus*), respectively. Furthermore, statistically significant effects on gill ATPase and plasma thyroxine in coho salmon occurred at arsenic trioxide concentrations that were one-tenth to one-third the concentration causing a decrease in a biologically important response (smoltification and downstream migration). These endpoint comparisons further support the recommendation of Mehrle and Mayer [6] that physiological and biochemical responses be related to important whole-animal responses (e.g., survival, growth and reproduction). Cellular or biochemical changes in organisms that are indicative of exposure to toxicants may or may not be precursors of morbidity [19].

The survival endpoint is more cost- and labor-efficient than most other measurements conducted in chronic toxicity tests. Although chemical intoxication is not generally characterized by only one endpoint, such as survival (different effects may be due to different modes of action), survival measurements appear to be adequate for estimating NOECs within the initial stages of chronic toxicity testing for hazard assessments. However, a factor of at least 0.2 (0.13 to 0.22) should be applied to the estimated NOEC for survival to include other biologically significant effects that may more appropriately describe the state of intoxication.

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