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QUANTITATIVE STRUCTURE-ACTIVITY RELATIONSHIPS FOR CHRONIC TOXICITY OF PHENOL, P-CHLOROPHENOL, 2,4-DICHLOROPHENOL, PENTACHLOROPHENOL, P-NITROPHENOL AND 1,2,4-TRICHLOROBENZENE TO EARLY LIFE STAGES OF RAINBOW TROUT (*Oncorhynchus mykiss*)

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Quantitative Structure-Activity Relationships for chronic toxicity of phenol, p-chlorophenol, 2,4-dichlorophenol, pentachlorophenol, p-nitrophenol, and 1,2,4-trichlorobenzene to early life stages of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Rainbow trout were exposed to waterborne phenol, p-chlorophenol, 2,4-dichlorophenol, p-nitrophenol or 1,2,4-trichlorobenzene for 85 days. This period included full egg development from the day of fertilization, plus hatching, yolk resorption and four weeks of feeding as freely-swimming fry. The primary effects of exposure were to reduce growth rate and to increase mortality rate. Growth inhibition was the most sensitive response since it occurred at exposure levels equal to or lower than those that increased mortality rates. Changes in development rate, growth efficiency and percent moisture were also observed after exposure to some chemicals. Changes within experiments due to treatment effects were generally larger than observed variations of control responses compared among experiments. Threshold exposure concentrations for the chronic toxicity of each chemical were calculated from regressions of the responses against the logarithm of exposure concentrations. The threshold was that concentration predicted to change a response by 25% relative to the control responses. The order of toxicity based on these thresholds was phenol < p-nitrophenol < p-chlorophenol < 2,4-dichlorophenol < 1,2,4-trichloro-benzene. Thresholds for pentachloro-phenol were also calculated from a published study of chronic toxicity to give a larger data base. These data defined a Quantitative Structure-Activity Relationship (QSAR) between the logarithm of threshold effect concentrations and the logarithm of the octanol-water partition coefficient. This QSAR was parallel to a QSAR for the acute lethality of these same chemicals. Since the ratio of the slopes of the two QSARs was 0.10, the data suggests that there is a constant ratio of about 0.10 between chronic and acute toxicity.

RESUME

Des truites arcs-en-ciel ont été exposées pendant 85 jours au phénol, p-chlorophénol, 2,4-dichlorophénol, p-nitrophénol ou 1,2,4-trichlorobenzène. Pendant cette période, il y avait succesivement le développement des oeufs, l'éclosion, la résorption du vitellin, et l'alimentation des jeunes pendant quatre semaines avec une nourriture artificielle. Les effets principaux étaient une réduction du taux de croissance et une augmentation du taux de mortalité. L'inhibition de croissance était la réponse la plus sensible, parce qu'elle arrivait à des niveaux d'exposition équivalents ou inférieure à ceux qui changeaient les taux de mortalité. Quelquefois, il y'avait d'autres réponses comme un changement de la vitesse de développement, de l'efficacité de croissance, et du pourcentage d'humidité dans les tissus. En genéral, les changements dus aux produits chimiques étaient plus grands que les variations normales mesurées entre les expériences. Les seuils d'exposition associés avec la toxicité chronique ont été calculés par des régressions entre les réponses et les logarithmes des concentrations de chaque composé chimique testé. Les seuils étaient les concentrations estimées pouvant induire un changement de 25 % relativement à la réponse du témoin. L'ordre de toxicité indiqué par les seuils était phénol < p-nitrophénol < p-chlorophénol < 2,4-dichlorophénol < 1,2,4-trichlorobenzène. De plus, pour avoir une base de données plus large, les seuils pour les réponses au pentachlorophénol ont été calculés à partir d'une étude déjà publiée. Ces données ont defini une Relation Quantitative entre la Structure et la Toxicité (RQST), c'est-à-dire, entre le logarithme des seuils de concentration et le logarithme du coefficient de distribution des composées chimiques entre le n-octanol et l'eau. Ce RQST était parallèle à une RQST calculée pour la toxicité létale aigue des mêmes produits chimiques. Les deux RQSTs suggèrent qu'il y a un rapport constant de 0.10 entre la toxicité chronique et la toxicité aigue, parce que le rapport entre les pentes était de 0.10.

INTRODUCTION

An important part of water quality management is the protection of aquatic species from toxic chemicals, particularly from the insidious effects of chronic exposures. Water quality criteria have been established to define those levels of chemical exposure that are "safe", based on literature reviews of controlled laboratory experiments. However, for most of the estimated 70,000 chemicals in common use, aquatic toxicity data are limited to acute toxicity tests (van Leuwen *et al.* 1990). Data from full life-cycle tests with long-lived species are even more scarce due to the high cost of extended tests. Since many organic chemicals already contaminate aquatic food chains (Hesselberg and Seelye 1982), extensive impacts may occur before environmental limits can be established.

The need to rapidly identify potentially hazardous chemicals and to protect sensitive species has engendered a variety of strategies, including truncated chronic toxicity tests (Macek and Sleight 1977; McKim 1977) and predictive models of toxicity. Models may be based on ratios of acute and chronic toxicity or on Quantitative Structure-Activity Relationships (QSARs, Veith *et al.* 1983).

Truncated chronic studies test embryogenesis or larval development, often the most sensitive stage of an organism's life-cycle. Hence early life stage studies may predict toxicity during full life-cycle tests (Macek and Sleight 1977; McKim 1977), although their accuracy is quite variable (Suter 1990).

Chronic toxicity and criteria to limit chemical levels in water have often been estimated by "Application Factors". These are fixed ratios between chronic and acute toxicity (usually 0.1 or 0.01), based on the assumption that the ratio is invariate. While this approach was acceptable in the past when data were limited (e.g. NAS/NAE 1972), modern practice demands a higher level of proof. Furthermore there is no guarantee of a fixed relationship between acute and chronic toxicity because: (a) chronic toxicity may arise from different mechanisms of toxicity; (b) metabolic detoxification can be induced during chronic exposure; and (c) acute toxicity is often measured near the limits of solubility.

QSARs compare measured toxicity to structural or physicochemical properties (molecular descriptors) of the test chemicals. QSARs based on tests of related chemicals with a systematic variation in molecular descriptors can predict the toxicities of other related but untested chemicals, thereby limiting testing effort.

QSARs have been successfully developed for the acute toxicity of chemicals to yeast (chloroanilines, Kwasniewska and Kaiser 1983), algae (azaarenes, aromatic amines and nitroaromatics, Schultz and Moulton 1984), *Daphnia* (hydrocarbons,

chlorinated hydrocarbons, oils, Bobra *et al.* 1984) and various species of fish (phenols, chlorophenols, aliphatics, benzenes, narcotics, Konemann 1981; Veith *et al.* 1983). The most useful molecular descriptor was the octanol-water partition coefficient (P) which describes the relative distribution of chemicals between water and n-octanol. The slopes of QSARs are very similar among studies, indicating that the characteristics most relevant to toxicity are chemical solubility and ability to penetrate lipid membranes. Measures of log P effectively mimic the partitioning of chemicals from water into lipids of aquatic biota.

Although embryo/larval studies are less expensive than full life-cycle tests, they still require long testing times; there are few QSARs based on truncated tests (anilines, chlorobenzenes, van Leuwen *et al.* 1990). If the potential of QSARs is to be realized and successfully applied to chemical control, we must test the assumption that QSARs based on acute toxicity predict chronic toxicity.

We measured the toxicity to embryos, larvae and fry of rainbow trout (*Oncorhynchus mykiss*) of a group of aromatic compounds chosen to give a wide range of water solubilities, partition coefficients, and acute toxicities. Estimated threshold effect concentrations were used to develop a QSAR with log P. We tested the hypothesis of a fixed relationship between acute (96 hour LC50s) and chronic toxicity by comparing the slopes of their respective QSARs.

Finally, since a standard test protocol for rainbow trout was not available, we examined the variability of control responses among toxicity tests.

MATERIALS AND METHODS

DESIGN

Rainbow trout eggs were exposed to six concentrations (including control) of each test chemical: phenol (PH); p-nitrophenol (NP); p-chlorophenol (CP); 2,4 dichlorophenol (DCP); and 1,2,4, trichlorobenzene (TCB). The concentration range spanned an order of magnitude from 0.05 to 0.5 of the 96-hr LC50. Increments of concentration were equal on a logarithm scale, on the assumption that each successive increment of response requires a doubling of the exposure intensity, according to Beer's law. The duration of each test was from the day of fertilization, through hatch and yolk sac resorption, to four weeks of feeding, a total of 85 days (Figure 1).

The life stages exposed were embryos (eggs), larvae (newly-hatched fish with obvious yolk sacs - also known as alevins or sac-fry), and fry (freely-swimming juveniles that feed exogenously). Larvae that have absorbed their

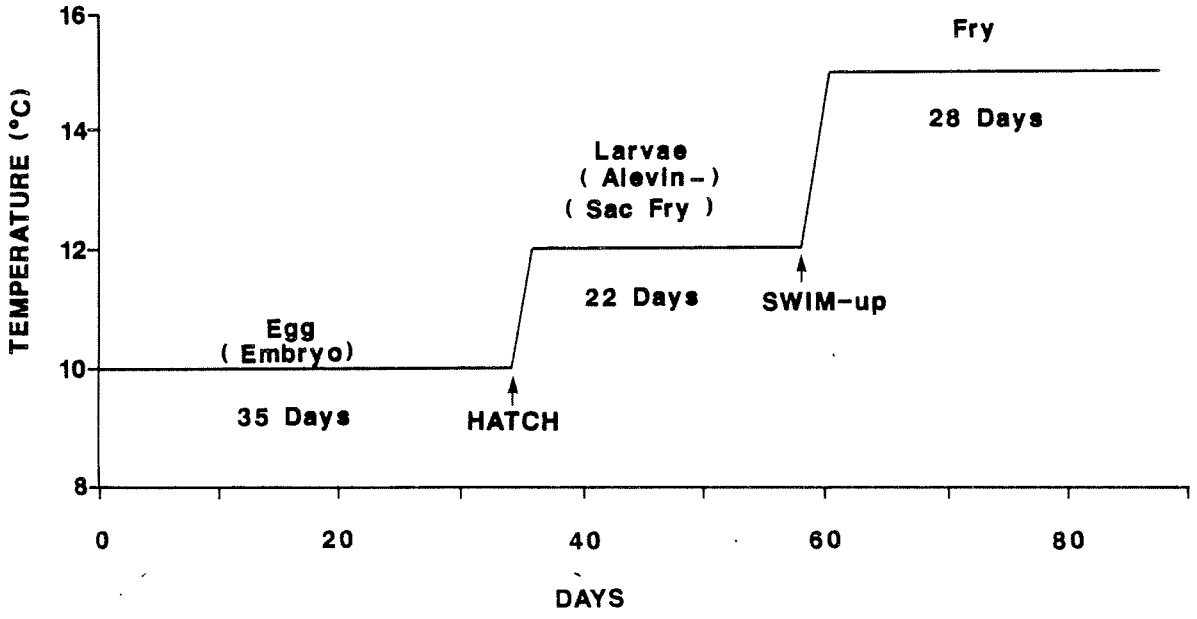

Figure 1. The temperature regime and duration of each life stage during rainbow trout embryo-larval-fry tests.

yolk sacs and that leave the bottom to begin feeding are called swim-up fry. Temperatures were maintained at 10°C for egg development, 12°C for yolk resorption and 15°C for fry growth (Figure 1).

Each experiment was replicated three times for a total of 18 tanks. Each replicate within any experiment received the fertilized eggs from a different female, and the order of chemical concentrations was randomized within each 6 tank replicate. Measurements of toxicity included hatching success, mortality, rate of development, prevalence of deformities and growth.

CHEMICALS

The test chemicals were either purchased as highly purified standards or repurified according to the methods listed (Table 1).

The test chemicals were added to water directly (TCB) or as aqueous solutions (PH, NP, CP, DCP) by a microlitre syringe pump linked to a diluter (Mount and Brungs 1967). An electronic timing device controlled both the pump and a solenoid valve supplying water to the diluter.

The dilutions were set for 0 (control), 10, 18, 32, 56 and 100 percent of the maximum concentration. The flow ranged from 300 to 600 mL per minute at each concentration, and the output of each cell was split three ways before distribution to replicate exposure tanks. The exposure tanks were glass aquaria measuring about 40 cm long by 20 cm wide by 20 cm high for a total volume of about 16 L, maintained at 14 L by a standpipe. Flow rate per tank ranged from 125 to 225 mL per minute, giving a 95% molecular replacement time of 3 to 5.5 hours (Sprague 1973). At a desired loading rate of 0.5 g of fish per litre of water per day, the flow limited total biomass per tank to about 90 to 162 g.

The concentrations of chemicals at each exposure level were measured daily by UV absorption spectrophotometry using standards prepared daily in control aquarium water. Levels of TCB were determined by gas-liquid chromatography after solvent extraction.

WATER

All experiments used water from Lake Ontario, delivered to the lab as a dechlorinated municipal supply. Hardness was 135 mg/L as $CaCO_3$ and alkalinity 80 mg/L as $CaCO_3$. Flow rates, dissolved oxygen, pH and conductivity measured during the experiments are summarized in Table 2. Detailed analyses of other characteristics are reported in Hodson *et al.* (1980).

TABLE 1

Properties and acute toxicity of the test chemicals.

CHEMICAL	MOLECULAR WEIGHT	WATER SOLUBILITY (mM)	LOG PARTITION COEFFICIENT[a] (LOG P)	LC50 FOR RAINBOW TROUT[b] (uM)	SOURCE[c]	LOT NUMBER	REFINING TECHNIQUE
phenol	94.1	>1000[d]	1.49	103	BDH	07541	---
p-nitrophenol	139.1	36[e]	1.91	57	A	HE5518HE	From ether-hexane
p-chlorophenol	128.6	210[f]	2.42	14.8	BDH	2450950	From hexane
2,4-dichlorophenol	163.0	38.0[g]	3.08	16.0	E	A4A	From hexane
1,2,4-trichlorobenzene	181.5	0.19[g]	4.26	8.3[h]	A	CE5814	Redistillation
pentachlorophenol	266.4	0.036[d]	5.12	0.6	F	31145-382	From hexane-toluene

a From Hansch and Leo, 1979.

b From Hodson et al. 1984.

c A, Aldrich Chemical Company; BDH, BDH Chemicals; C, Caledon Laboratories; E, Eastman Kodak; F, Fluka Chemical Corporation.

d From Jones, 1981.

e Verschueren, 1983.

f From Suntio et al. 1988.

g From Yalkowski et al. 1979.

h From US EPA, 1980.

FISH

Rainbow trout eggs from three females were purchased for each test from Goosen's Rainbow Ranch, Otterville, Ontario. After fertilization, they were taken to the lab in thermos jugs and the chemical exposure started. About 200-300 eggs were allocated impartially to each exposure tank for a total of 3500-4500 eggs per experiment. Each of the six tanks within a replicate received eggs from the same adult female.

Within each tank, eggs were held in kitchen sieves with nylon screen bottoms and solid sides. These containers encircled the standpipe drain so that water leaving the tank flowed up through the eggs before spilling over the top of the standpipe. A screen over the standpipe prevented the loss of larvae after hatch. Daily records were kept of dead eggs, sorted according to whether eye pigments were visible (eyed eggs), and whether mortality occured during the process of hatching. Temperature was recorded daily in each tank and cumulative total temperatures were used to estimate degree-days to hatch (the sum of daily temperatures).

The number of larvae hatched each day were counted and transferred from the egg baskets to the main tank to keep a record of daily and cumulative hatch. The number of degree-days to 50% hatch and from hatch to swim-up was estimated by probit analysis to give an index of the rate of development. The number of deformed larvae was also counted and expressed as a percentage of the total number hatched.

Yolk sac conversion efficiency was measured on a day when more than 20 larvae hatched. Ten were segregated and ten were immediately dissected to measure the separate wet and dry weights of the pooled larvae and yolk sacs. Dry weights were measured by drying to a constant weight at 60 C in a convection oven. The remaining ten fish developed for a further ten days in a separate egg incubation basket before being dissected as indicated. Yolk sac conversion efficiency was calculated as the ratio of weight gain of the larvae to weight loss of the yolk during the 10-day period (Hodson and Blunt 1986). Percent moisture of the dissected larvae was also calculated as an index of osmoregulation and catabolism.

When the yolk of each fish was almost completely used and the fish began to swim towards the surface, feeding was initiated with a salmonid "starter" diet (Martin's Feed Mills, Elmira, Ontario). Feeding was done gradually and carefully to minimize wastage; feces and uneaten food were removed daily. When feeding was successful, the fish were counted and weighed live in water. This established the initial weight and biomass of feeding fry. Weighing was repeated at weekly intervals. When total biomass of a tank approached the recommended loading rates, 50% of the fish were removed and discarded. Four weeks after

TABLE 2

Range of conditions in exposure tanks throughout the toxicity tests.

Chemical	Flow (ml/min)	Oxygen (mg/L)	pH	Conductivity (µmhos/cm²)
phenol	196-206	11.0-11.5	7.97-8.02	244-245
p-nitrophenol	160-196	9.4-10.9	7.83-7.96	244-245
p-chlorophenol	195-205	11.6-11.8	8.08-8.10	242-243
2,4-dichlorophenol	163-171	10.0-10.9	7.87-8.00	246-250
1,2,4-trichlorobenzene	125-134	11.5-11.7	8.09-8.15	233-235
pentachlorophenol	225	8.6-11.0	7.87-8.08	267-283

feeding began, ten fish from each tank were removed to measure pooled wet and dry weights and percent moisture.

Mortality of fish (both larvae and fry combined) was expressed as a percent of the total in each tank at hatch. To avoid bias caused by removing fish, the total number of fish-days (cumulative number of fish in any tank on each day) was also calculated and mortality expressed as the number per 1000 fish-days.

STATISTICS

Changes in each measurement were assessed by analyses of variance (ANOVA) and post-hoc comparisons of treatment means with controls. Threshold concentrations for chemical effects were estimated from exposure-response curves defined by linear regression analysis. QSARs for chronic toxicity were developed by comparing threshold exposure levels to octanol-water partition coefficients by linear regression. All tests were at the 0.05 probability level.

Prior to ANOVA, Shapiro-Wilk's normality test, Bartlett's homogeneity of variance test, plots of residuals, and plots of means vs variances were used to identify serious violations of assumptions about normality and homogeneity of variance (Sokal and Rohlf 1981).

As eggs within each replicate were derived from a different female, the responses of fish to chemical exposure were tested by a randomized complete block design for ANOVA (Steel and Torrie 1960). A two-tailed Dunnett's test (Steel and Torrie 1960) determined which treatment means were different from control. For unequal sample sizes, we used a Dunnett's test with the Kromer modification (Day and Quinn 1989). The minimum difference from control that could be declared significant by Dunnett's test is a function of the measured variance and was calculated for each parameter.

Statistically significant responses may occur coincidentally at least once in every 20 tests at the 0.05 probability level. Since 30 tests were carried out per experiment there was a high risk of "false positives". To minimize this risk, we did not consider a response significant unless it met the following criteria: 1) a significant "F" statistic for treatments from the ANOVA; 2) a monotonic exposure-response relationship; isolated or irregular responses in the middle of an exposure range were not accepted; and 3) a change from the control response by 25% or more.

For responses meeting these criteria, the threshold effect concentration was defined as that concentration associated with a 25% change from control values, as suggested by the US EPA (1989). The threshold concentration was estimated by three methods. The first calculated the geometric mean

of the LOEC, defined as the Lowest Observed Effect Concentration causing a change from the control response of 25% or more, and the NOEC, the next lowest or No Observed Effect Concentration. The NOEC and LOEC define a range equivalent to the MATC (Maximum Acceptable Toxicant Concentration, McKim 1985). The threshold is the geometric mean or the mid-point of this range on a log scale.

The second method calculated the simple linear regression relating responses to the logarithm of exposure concentrations. The threshold was interpolated as that concentration associated with a 25% change in response from control values. Parameters were tested only if they met the following criteria: 1) significant treatment effects demonstrated by the ANOVA; 2) largest response at least 25% greater than control; 3) and responses at a minimum of three exposure levels.

The third technique was linear interpolation by the "Bootstrap Method" (Norbert-King 1988; US EPA 1989). All calculations were performed by the "BOOTSTRP ICp" software package (developed by Battelle Columbus, in cooperation with the US EPA 1989). This method assumes that responses are monotonically decreasing (the mean response for each higher concentration is less than or equal to the mean response for the previous concentration) and that responses follow a piece-wise linear response function (a linear response from one concentration to the next). If means do not decrease monotonically, the responses are "smoothed" by averaging adjacent means.

QSAR ANALYSIS

A chronic QSAR was developed by simple linear regression of log threshold concentrations determined by the regression method versus log P. An acute lethality QSAR was similarly developed from the published LC50s for these chemicals for rainbow trout (Hodson *et al.* 1984). The homogeneity of QSAR slopes were compared by a t-test (Sokal and Rohlf 1981).

PENTACHLOROPHENOL

The pentachlorophenol (PCP) data in this report were derived from a previous study (Hodson and Blunt 1981). The methodology of the PCP experiment was the prototype for the current work, but the temperature regime (12°, 15° and 20° for egg development, yolk sac resorption and fry growth respectively), number of treatments (4) and number of replicates (2) differ from the current protocol. The PCP data were the response means and summary statistics from a factorial ANOVA that tested concentration, temperature and concentration-temperature interaction effects ($P \leq 0.01$).

No post-hoc comparisons of treatment means with controls were possible. However LOEC values

could be estimated for responses which exhibited significant concentration effects, and which changed only at the highest exposure level. The 25 percent effect threshold concentrations for the PCP data were interpolated from linear regressions relating response to the logarithm of exposure concentration.

RESULTS

WATER CONDITIONS

The routine water analyses demonstrated that water quality in each test remained constant throughout the tests. There were no differences among treatment and control conditions, other than in the level of added chemicals (Table 2). The levels of added chemicals fluctuated slightly and separate average concentrations are reported for each phase (eggs, larvae, fry) of each test to ensure accurate estimation of thresholds (Tables 4-8). Spectrophotometric determination of CP and DCP proved difficult due to interferences. Gas liquid chromatography, after solvent extraction and derivitization, indicated that measured concentrations were close to nominal. However, frequent determinations proved too time consuming and costly. Therefore, reported concentrations for CP and DCP (Tables 6 and 7) are nominal values.

Daily records indicated that water temperatures throughout all tests remained within 1°C of target values.

CONTROL RESPONSES

Mean control responses (Table 3) indicated a considerable variation among experiments in the percent mortality of all eggs, which ranged from 2.5 to 24.6%. This range appears to reflect variability in egg fertilization among experiments, as many eggs were unfertilized (no obvious cell division as shown by microscopic examination, lack of cloudiness, occurrence of large yellow oil droplets, no movement), and should not be considered true mortalities. Eyed eggs, those that developed sufficiently to be easily recognized as living, showed a much smaller range of control mortality, from 0 to 10.4%. One control replicate in the CP experiment exhibited a 60% mortality for eyed eggs, compared to less than 3% for the remaining two replicates. The same replicate had a 75% mortality for fry. Tank contamination was suspected and this control replicate was removed from the analyses.

Percent mortality during hatch was also much more consistent (0-7.3%). The inverse of percent mortality, percent hatch (i.e. the percentage of eggs surviving to hatch), showed the same ranges of variability on a total and eyed egg basis as percent mortality of eggs. Average development rates for eggs varied from 340 to 381 degree-days, corresponding to

average temperatures during egg development of 9.8 to 9.6°C; development rate increased (fewer degree-days) with average incubation temperature.

At hatch, the weights of whole fish varied from 67.6 to 81.4 mg among experiments (Table 3), with a standard deviation equivalent to 7.8% of the overall control mean. When considered by components, the larvae alone also had a relative standard deviation of 7.8%, with the yolks accounting for the missing weight and having a standard deviation of 8.7%. Percent moisture was not as variable (SD = 1.4%).

Percent mortality of sac fry and development rate were quite consistent (Table 3) but data for NP and DCP were missing. Percent deformities (bug eye, blue sac, corkscrew) were somewhat more variable, with a standard deviation of 5.9%.

At 10 days post-hatch, the average weight of whole fish varied from 83.2 to 107.2 mg among experiments (Table 3); the standard deviation was 10.3%, compared to 7.8% at hatch. However, the weights of larvae alone at 10 days post-hatch and the weights of yolk had standard deviations of 16.7% and 33.0% respectively, compared to their hatch weight counterparts of 7.8% and 8.7%. Increasing variability indicates possible differences in growth rates and growth efficiencies among experiments. The conversion efficiency of yolk exhibited a standard deviation of 20.2%. Percent moisture varied little except for DCP, which had a mean value of 75%, about 12% lower than that of larvae in other experiments. Within-group variations for the wet and dry weights indicated that dry weight was overestimated in one replicate, which biased the mean percent moisture.

During feeding, percent mortality of fish ranged from 6.6% to 20.0% or 1.97 to 5.00 mortalities per 1000 fish-days (Table 3). These values represent both larval and fry mortality combined. Total number of fish-days varied considerably (range: 4375 to 9653), demonstrating a standard deviation 26.4% of the overall control mean. Fry weight at 4 weeks post swim-up also differed considerably among experiments with a standard deviation of 27.7%. Weight differences for fry did not follow the same relative order as larvae due to differences in growth rates among experiments.

Overall, variability in measured parameters increased with the stage of development as different rates of development were expressed.

RESULTS OF ANALYSIS OF VARIANCE AND DUNNETT'S TEST

Since each experiment included many measurements, only those that showed changes are discussed in the text, but all results are reported in the tables. A change is defined

TABLE 3

Mean control responses of rainbow trout to test compounds.

	n							
	3 PH	3 NP	2 CP	3 DCP	3 TCB	Mean	Standard Deviation	Range
EGGS								
% Mortality of all eggs	11.6	24.0	2.5	11.2	24.6	14.8	9.4	2.5 - 24.6
% Mortality of eyed eggs	4.6	3.0	0.0	0.6	10.4	3.7	4.2	0.0 - 10.4
% Hatch of eyed eggs	95.4	97.0	100.0	99.4	89.6	96.3	4.2	89.6 - 100.0
% Died while hatching	3.5	2.0	0.0	0.6	7.3	2.7	2.9	0.0 - 7.3
% Hatch of all eggs	88.4	76.0	97.5	88.6	75.4	85.2	9.4	76.0 - 97.5
Degree days to hatch	340.0	381.0	365.0	361.0	347.0	359.0	16.0	340.0 - 381.0
Wet weight of "larvae only" at hatch (mg)	27.1	24.6	30.6	27.3	27.1	27.3	2.1	24.6 - 30.6
Dry weight of "larvae only" at hatch (mg)	3.8	2.8	4.2	4.2	3.5	3.7	0.6	2.8 - 4.2
Wet weight of yolk sac at hatch (mg)	41.8	43.0	50.8	48.0	42.7	45.3	3.9	41.8 - 50.8
Dry weight of yolk sac at hatch (mg)	19.0	17.7	23.6	23.0	19.8	20.6	2.6	17.7 - 23.6
Wet weight of larvae and yolk sac at hatch (mg)	69.0	67.6	81.4	75.2	69.8	72.6	5.7	67.6 - 81.4
Dry weight of larvae and yolk sac at hatch (mg)	22.9	22.5	27.8	27.3	23.4	24.8	2.5	22.5 - 27.8
% Moisture at hatch	85.8	88.0	86.2	84.8	87.0	86.4	1.2	84.8 - 88.0
LARVAE								
% Mortality of larvae	3.0	7.0	4.0	3.7	7.2	5.0	2.0	3.0 - 7.2
% Deformities	5.1	0.3	13.2	0.2	10.1	5.8	5.8	0.2 - 13.2
Degree-days from hatch to swim-up	101.2	---	99.2	---	103.4	101.3	2.1	99.2 - 103.4
Wet weight of "larvae only" at 10 days (mg)	67.7	47.4	72.6	57.8	71.0	63.3	10.6	47.4 - 72.6
Dry weight of "larvae only" at 10 days (mg)	9.5	7.6	9.9	15.4	10.1	10.5	2.9	7.6 - 15.4
Wet weight of yolk sac at 10 days (mg)	24.2	35.8	32.0	36.8	24.8	30.7	6.0	24.2 - 36.8
Dry weight of yolk sac at 10 days (mg)	12.1	16.2	15.9	16.5	12.5	14.6	2.2	12.1 - 16.5
Wet weight of larvae and yolk sac at 10 days (mg)	91.9	83.2	105.6	107.2	95.8	96.7	9.9	83.2 - 107.2
Dry weight of larvae and yolk sac at 10 days (mg)	21.7	23.8	25.8	31.9	22.7	25.2	4.0	21.7 - 31.9
Yolk sac conversion efficiency (wet)	2.31	3.26	2.28	2.08	2.46	2.48	0.5	2.08- 3.26
Yolk sac conversion efficiency (dry)	0.83	1.59	0.73	1.75	0.90	1.16	0.5	0.83- 1.75
% Moisture at 10 days	85.9	84.0	86.6	75.0	85.7	83.4	4.8	75.0 - 86.6

TABLE 3 (continued)

Mean control responses of rainbow trout to test compounds.

	n							
	3 PH	3 NP	2 CP	3 DCP	3 TCB	Mean	Standard Deviation	Range
FRY								
% Mortality of fish	7.9	20.0	15.2	6.6	13.1	12.6	5.5	6.6 - 20.0
Mortality per 1000 fish-days	1.97	5.00	3.40	2.00	3.71	3.22	1.3	1.97 - 5.00
Total number of fish-days	7090	7281	9653	6958	4375	7071	1870	4375 - 9653
Wet weight at week 4 post swim-up (mg)	320	452	491	704	539	501	140	320 - 704
Dry weight at week 4 post swim-up (mg)	61.7	87.4	95.4	---	110	88.6	20.2	61.7 - 110
% Moisture at week 4 post swim-up	80.8	81.0	80.6	---	79.5	80.5	0.7	79.5 - 81.0

PH, phenol; NP, p-nitrophenol; CP, p-chlorophenol; DCP, 2,4-dichlorophenol; TCB, 1,2,4-trichlorobenzene.

"-" = no data.

as a statistically significant difference among the treatment means.

Phenol

No effects of PH on eggs were observed during development but one test replicate, for the 30 uM level of PH, had a mortality rate of 97% for eggs due to a sudden rise in tank water temperature. The number of replicates at this exposure level was reduced to two (Table 4).

At hatch, significant decreases were observed in the mean weight of "larvae-only" relative to control. Decreases followed an exposure-response trend with a 39% reduction in weight at the highest exposure concentration (Figure 2). Although the weights of whole fish (larva plus yolk) decreased relative to control (a 14% reduction at the highest test concentration), none were statistically significant. At the highest test concentration, percent moisture at hatch increased a maximum of 2% relative to the control mean. Larvae also demonstrated a significant increase in percent mortality (Table 4), by about 17% relative to control at the highest test concentration.

Weights of PH-exposed larvae decreased significantly at 10 days post-hatch. Decreases followed an exposure-response relationship terminating in a 44% reduction in weight relative to controls. Although yolk sacs at 10 days post-hatch were about 26% larger than controls at the highest test concentration, the change was not significant due to large within-group variations. Significant reductions in the weights of whole fish (larva plus yolk) were observed, with the mean weight at the highest test concentration 25% lower than control.

A 24% reduction in yolk sac conversion efficiency was observed at the highest test concentration, but responses were erratic: two responses at mid-range concentrations increased relative to control and changes were not statistically significant. In contrast, percent moisture of larvae at 10 days post-hatch increased by only 1% relative to control and the change was statistically significant.

Fry percent mortality followed an obvious exposure-response relationship (Table 4) terminating in a 65% increase in mortality compared to control. The corresponding increase in mortality per 1000 fish-days was from 2.0 to 25.4.

The mean weight of fry decreased by about 27% at the second highest PH concentration, but was only 15% lower than control at the highest concentration. These changes were not statistically significant due to large within-group variations, but percent moisture increased significantly by 3% at the highest test concentration.

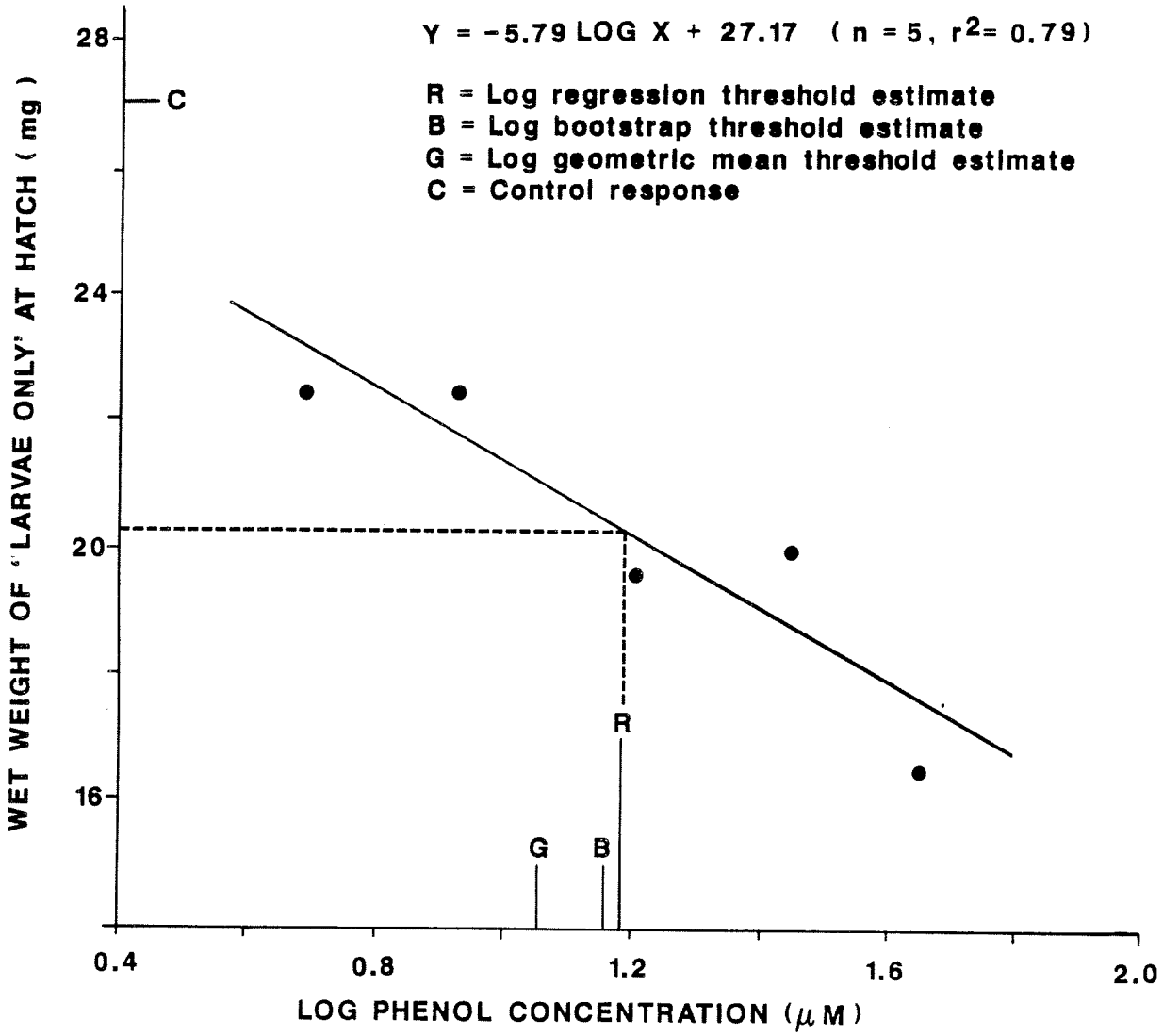

Figure 2. The effect of exposure to phenol on the wet weight of rainbow trout larvae at hatch. Threshold effect concentrations are shown for each statistical method of estimation.

TABLE 4

Mean responses of rainbow trout to phenol exposure and results of analysis of variance and Dunnett's test.

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
EGGS											
phenol concentration (μM)	0.0	4.84	8.46	16.00	30.20	44.00					
% Mortality of eyed eggs	4.6	3.1	2.7	3.6	2.8	7.6	2.88	2.35	1.24		
% Hatch of eyed eggs	95.4	96.4	97.3	96.4	97.2	92.4	2.88	2.35	1.24		
% Died while hatching	3.5	1.4	0.5	0.6	0.3	1.7	2.49	2.03	0.54		
Degree days to hatch	340.2	347.0	341.2	348.6	352.4	350.6	5.24	4.28	2.46		
Wet weight of "larvae only" at hatch (mg)	27.1	22.5	22.9	19.5*	20.0*	16.6*	1.95	1.59	10.14	5.06	19
Dry weight of "larvae only" at hatch (mg)	3.8	2.9*	2.9*	2.6*	2.3*	2.0*	0.23	0.19	20.54	0.60	16
Wet weight of yolk sac at hatch (mg)	41.8	38.3	40.2	39.6	40.0	43.9	3.95	3.23	0.73		
Dry weight of yolk sac at hatch (mg)	19.0	17.9	18.6	18.4	18.6	16.9	2.45	2.00	0.27		
Wet weight of larvae and yolk sac at hatch (mg)	69.0	60.8	63.1	59.1	60.0	60.5	4.08	3.34	2.29		
Dry weight of larvae and yolk sac at hatch (mg)	22.9	20.8	21.5	21.0	20.9	18.9	2.46	2.01	0.83		
% Moisture at hatch	85.8	87.4	87.2	86.6	88.5*	87.8*	0.71	0.58	4.45	1.84	2
LARVAE											
phenol concentration (μM)	0.0	4.63	7.66	14.60	29.50	55.30					
% Mortality of larvae	3.0	2.2	3.0	4.2	9.5*	20.8*	2.44	1.99	25.78	6.33	6
% Deformed	5.1	1.6	4.1	1.8	5.4	2.9	3.07	2.51	0.77		
Degree days from hatch to swim-up	101.2	101.8	101.7	101.4	101.0	102.8	1.25	1.02	0.69		
Wet weight of "larvae only" at 10 days (mg)	67.7	60.3	58.7	54.5*	52.5*	37.9*	4.43	3.62	15.17	11.51	17
Dry weight of "larvae only" at 10 days (mg)	9.5	8.3	8.0	7.5*	7.4*	5.0*	0.66	0.54	15.66	1.72	18
Wet weight of yolk sac at 10 days (mg)	24.2	21.8	26.0	26.2	24.4	30.5	4.10	3.35	1.49		
Dry weight of yolk sac at 10 days (mg)	12.1	10.9	13.0	13.2	12.2	14.9	2.11	1.72	1.19		
Wet weight of larvae and yolk sac at 10 days (mg)	91.9	82.1*	84.7*	80.7*	76.8*	68.4*	2.75	2.25	24.04	7.16	8
Dry weight of larvae and yolk sac at 10 days (mg)	21.7	19.3	20.9	20.8	19.6	19.9	1.73	1.42	0.79		
Yolk sac conversion efficiency (wet)	2.31	2.30	2.78	3.25	2.07	1.64	0.78	0.64	1.53		
Yolk sac conversion efficiency (dry)	0.83	0.79	1.05	1.50	0.86	0.63	0.84	0.68	0.80		
% Moisture at 10 days	85.9	86.2*	86.5	86.3	86.0	86.8*	0.22	0.18	11.18	0.60	1

TABLE 4 (continued)

Mean responses of rainbow trout to phenol exposure and results of analysis of variance and Dunnett's test.

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
FRY											
phenol concentration (μM)	0.0	2.93	5.21	13.60	29.40	42.30					
% Mortality of fish	7.9	9.2	13.7	15.1	37.0*	73.0*	8.32	6.79	27.48	21.59	22
Mortality per 1000 fish-days	1.97	2.17	3.60	4.00	10.35*	25.40*	1.61	1.31	95.80	4.16	
Total number of fish-days	7090	10179	8072	8083	7393	4624	1131	1070	5.67	3403	48
Wet weight at week 4 post swim-up (mg)	319.7	311.5	302.8	311.3	234.0	271.1	93.90	76.72	0.29		
Dry weight at week 4 post swim-up (mg)	61.7	58.7	55.7	57.9	41.5	45.7	18.60	15.19	0.47		
% Moisture at week 4 post swim-up	80.8	81.1	81.6	81.4	82.2	83.4*	0.55	0.45	8.15	1.43	1

S, standard deviation calculated from pooled error variance; S_d, standard difference between means used to calculate D value for Dunnett's test; F, F value from ANOVA; D, minimum significant difference from control in original units as calculated by Dunnett's test; % diff., minimum significant percent difference from control.

* Treatment means significantly different from control by Dunnett's test.

p-Nitrophenol

For most responses, NP exposure had no effects on eggs (Table 5), but differences were observed for developmental rate. Two concentrations increased degree-days to hatch by up to 13%. Although an exposure-response trendwas evident, the two significant responses were not consecutive.

Weights, moisture and percent mortality of larvae at hatch, and between hatch and swim-up varied among treatments. However the variations with exposure were erratic and not significant.

Percent deformities varied minimally (range 0 to 2.59%) but replicates violated the assumption of homogeneity of variance and no statistical analyses were performed. Yolk sac conversion efficiencies also varied erratically. Although changes were significant by ANOVA, none were different from control by Dunnett's test.

For fry, an exposure-related increase in mortality was only 12% greater than control and was not considered significant (Table 5). However, mean weights of fry decreased by up to 51% significantly following an exposure-response relationship (Figure 3).

p-Chlorophenol

Reduced larval weights at hatch were the only measured responses of eggs to CP. Weights followed an exposure-response trend with a 17% decrease from control weights at the highest test concentration.

After hatch, mortality of larvae increased by 25% over control but only at the highest exposure level (Table 6). At this test concentration, larval weights at 10 days post-hatch decreased by 29% relative to controls (Figure 4), following an exposure-response relationship. Weights of whole fish at the highest test concentration also declined by 18% relative to controls but differences in yolk weights were not related to treatments.

At the highest test level, percent mortality of fry (Table 6) increased abruptly by 27%, corresponding to an increase in mortality per 1000 fish-days from 3.4 to 13.1. Weight at 4 weeks post swim-up was also reduced, by 38% relative to control. Percent moisture at 4 weeks post swim-up showed significant changes, but none were different from control.

2,4-Dichlorophenol

For eggs exposed to DCP, mean larval weight at hatch decreased significantly, following an exposure-response trend (Table 7). Weight reduction at the highest

test concentration was 27% from control. Percent moisture at hatch decreased significantly at the highest exposure level, by 3% relative to control.

After hatch, percent mortality of larvae exhibited a sharp monotonic rise, increasing by 70% at a mid-range test concentration (Table 7). Mortality levelled off at the final two test levels. There was an exposure-related decrease in larval weights at 10 days post-hatch. Weight reduction at the highest test concentration was 52% relative to control. Although yolk weight varied significantly, only weight at the lowest treatment was different from control. This was considered coincidental.

There was a sharp monotonic rise in percent mortality of fry (Table 7), which reached a plateau at a mid-range test concentration (Figure 5). Mortality increased by as much as 83% over control, corresponding to an increase in mortality per 1000 fish-days from 2.0 to 66.7. Total number of fish-days at the highest test concentration were reduced compared to control.

Fry weights at four weeks post swim-up were different from control at all test levels. Only three levels were tested as high mortality had depleted the number of fry at the two highest concentrations of DCP to less than 10 per tank. Weights were reduced by as much as 70% relative to mean control values (Figure 6).

1,2,4 -Trichlorobenzene

After exposure of eggs to TCB, the mean larval weights at hatch decreased relative to control (Table 8). The F-ratio for the analysis of variance was 2.94, slightly below the critical value of 3.33. However, Dunnett's test indicated that a mean weight reduction of 15% at the highest test concentration was significant. A yolk weight increase relative to control of 8% at the highest test concentration was also significant, but no differences were observed for weights of whole fish or percent moisture at hatch.

Larval mortality increased at the highest test concentration by 60% relative to the controls (Table 8). Percent deformities varied significantly from controls but with no obvious dose-response relationship; the result was considered coincidental.

Larval weights at 10 days post-hatch showed significant, monotonic reductions, culminating in a 41% decrease relative to control. Yolk weights increased over controls for all test levels, but only two responses were significant. Weights of whole fish decreased monotonically with increasing test concentrations. All weight losses were significant and culminated in a 21% weight reduction relative to controls. A 37% decrease from control of yolk sac conversion efficiency was observed at the highest exposure

TABLE 5

Mean responses of rainbow trout to p-nitrophenol exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
EGGS											
p-nitrophenol concentration (μM)	0.0	3.88	7.19	8.99	16.46	28.75					
% Mortality of eyed eggs	3.0	1.0	4.0	4.0	4.0	14.0	8.40	6.85	1.01		
% Hatch of eyed eggs	97.0	99.0	96.0	96.0	96.0	86.0	8.40	6.85	1.01		
% Died while hatching	2.0	0.0	2.0	3.0	2.0	10.0	6.40	5.22	0.36		
Degree days to hatch	381.0	398.0	411.0	431.0*	415.0	432.0*	17.30	14.12	3.96	44	12
Wet weight of "larvae only" at hatch (mg)	24.6	22.5	16.3	19.5*	18.7	18.1	5.50	4.49	1.37		
Dry weight of "larvae only" at hatch (mg)	2.8	4.3	2.7	2.6*	3.2	3.8	1.20	0.98	0.99		
Wet weight of yolk sac at hatch (mg)	43.0	42.6	39.9	39.6	43.4	42.5	3.60	2.94	0.81		
Dry weight of yolk sac at hatch (mg)	17.7	19.3	17.7	18.4	18.9	18.9	1.60	1.30	0.72		
Wet weight of larvae and yolk sac at hatch (mg)	67.6	65.1	56.2	59.1	62.1	60.6	6.70	5.47	1.02		
Dry weight of larvae and yolk sac at hatch (mg)	22.5	23.6	21.4	21.0	22.1	22.7	1.40	1.14	1.82		
% Moisture at hatch	88.0	82.0	83.0	86.6	83.0	79.0	4.00	3.26	1.60		
LARVAE											
p-nitrophenol concentration (μM)	0.0	2.73	5.97	7.19	13.80	21.57					
% Mortality of larvae	7.0	6.0	7.0	12.0	6.0	8.0	3.80	3.10	0.83		
% Deformed	0.34	0.34	0.43	1.48	0.0	2.59	1.27	1.04			
Degree days from hatch to swim-up	-	-	-	-	-	-					
Wet weight of "larvae only" at 10 days (mg)	47.4	48.0	42.4	38.1	43.2	42.9	13.80	11.26	0.22		
Dry weight of "larvae only" at 10 days (mg)	7.6	7.0	6.7	6.0	6.7	6.4	2.50	2.04	0.14		
Wet weight of yolk sac at 10 days (mg)	35.8	30.6	33.9	33.7	35.0	34.0	5.40	4.41	0.33		
Dry weight of yolk sac at 10 days (mg)	16.2	15.0	15.4	15.9	16.4	16.4	2.00	1.63	0.24		
Wet weight of larvae and yolk sac at 10 days (mg)	83.2	78.6	75.3	71.7	78.1	75.9	10.60	8.64	0.34		
Dry weight of larvae and yolk sac at 10 days (mg)	23.8	22.1	22.1	21.9	22.3	22.9	1.60	1.30	0.64		
Yolk sac conversion efficiency (wet)	3.26	2.71	3.72	1.90	2.14	1.83	0.99	0.81	1.41		
Yolk sac conversion efficiency (dry)	1.59	0.76	1.83	0.89	0.99	0.69	0.37	0.30	3.71	0.93	59
% Moisture at 10 days	84.0	85.0	84.0	84.0	85.0	85.0	1.40	1.14	0.27	0.60	1

TABLE 5 (continued)

Mean responses of rainbow trout to p-nitrophenol exposure and results of analysis of variance and Dunnett's test

	n						S	S_d	F	D	% diff.
	3	3	3	3	2	3					
FRY											
p-nitrophenol concentration (μM)	0.0	2.08	4.17	4.82	10.50	17.97					
% Mortality of fish	20.0	23.0	24.0	27.0	30.0	32.0	7.60	6.20	1.20	21.59	22
Mortality per 1000 fish-days	5.0	6.0	7.0	8.0	9.0	11.0	2.50	2.04	2.17	4.16	
Total number of fish-days	7281	8368	7850	6334	6260	5196	1140	930	3.20	3403	48
Wet weight at week 4 post swim-up (mg)	452.3	378.0	366.3	358.0	322.7*	221.3*	36.60	29.86	3.90	92.88	20
Dry weight at week 4 post swim-up (mg)	87.4	65.4	64.6	58.8	51.6	35.5*	14.20	11.59	4.41	36.04	41
% Moisture at week 4 post swim-up	81.0	83.0	83.0	84.0	84.0	84.0	1.70	1.39	1.64		

S, standard deviation calculated from pooled error variance; s_d, standard difference between means used to calculate D value for Dunnett's test; F, F value from ANOVA; D, minimum significant difference from control in original units as calculated by Dunnett's test; % diff., minimum significant percent difference from control.

* Treatment means significantly different from control by Dunnett's test.

"-" = no data.

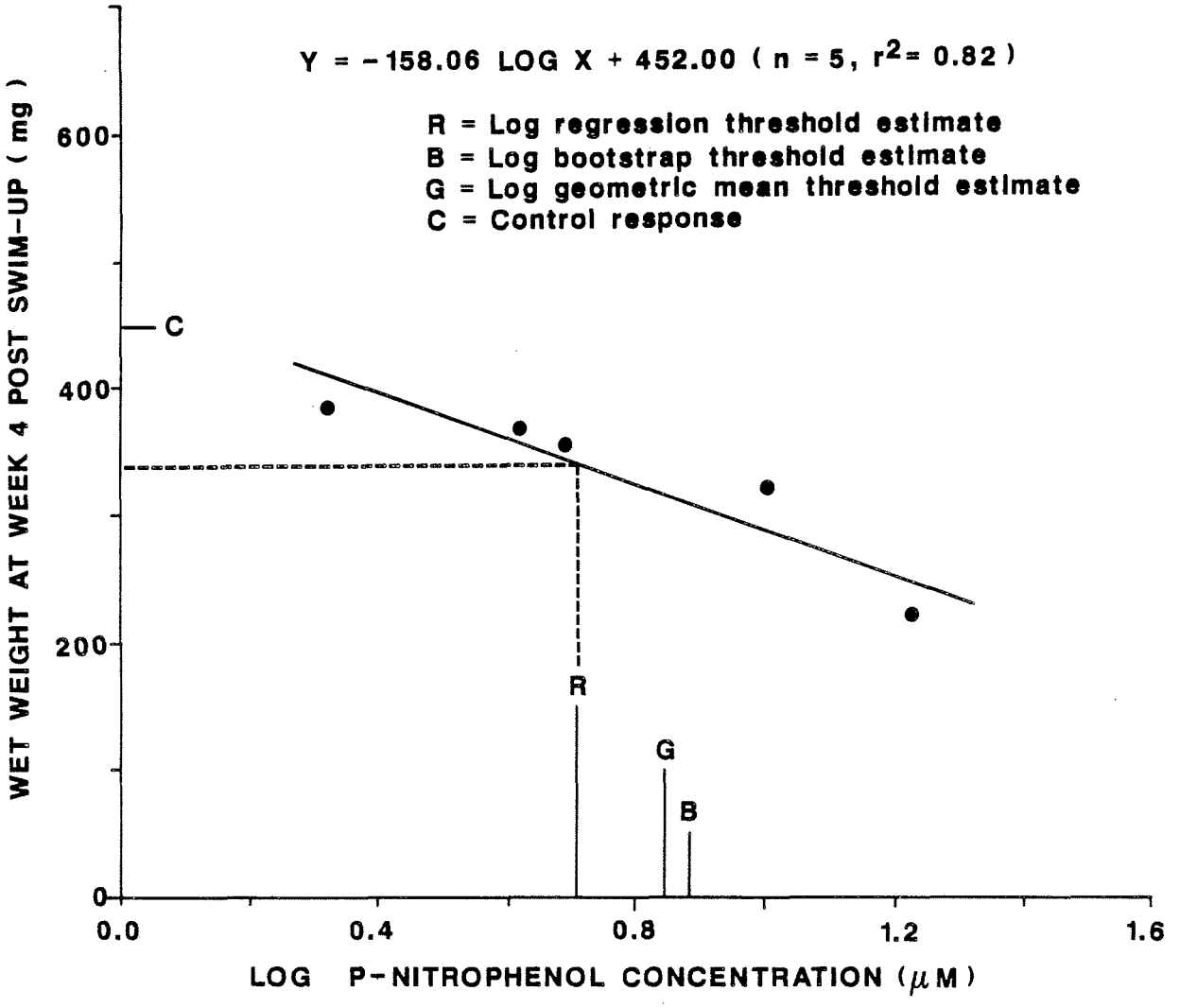

Figure 3. The effect of exposure to p-nitrophenol on the wet weight of rainbow trout fry after four weeks of feeding. Threshold effect concentrations are shown for each statistical method of estimation.

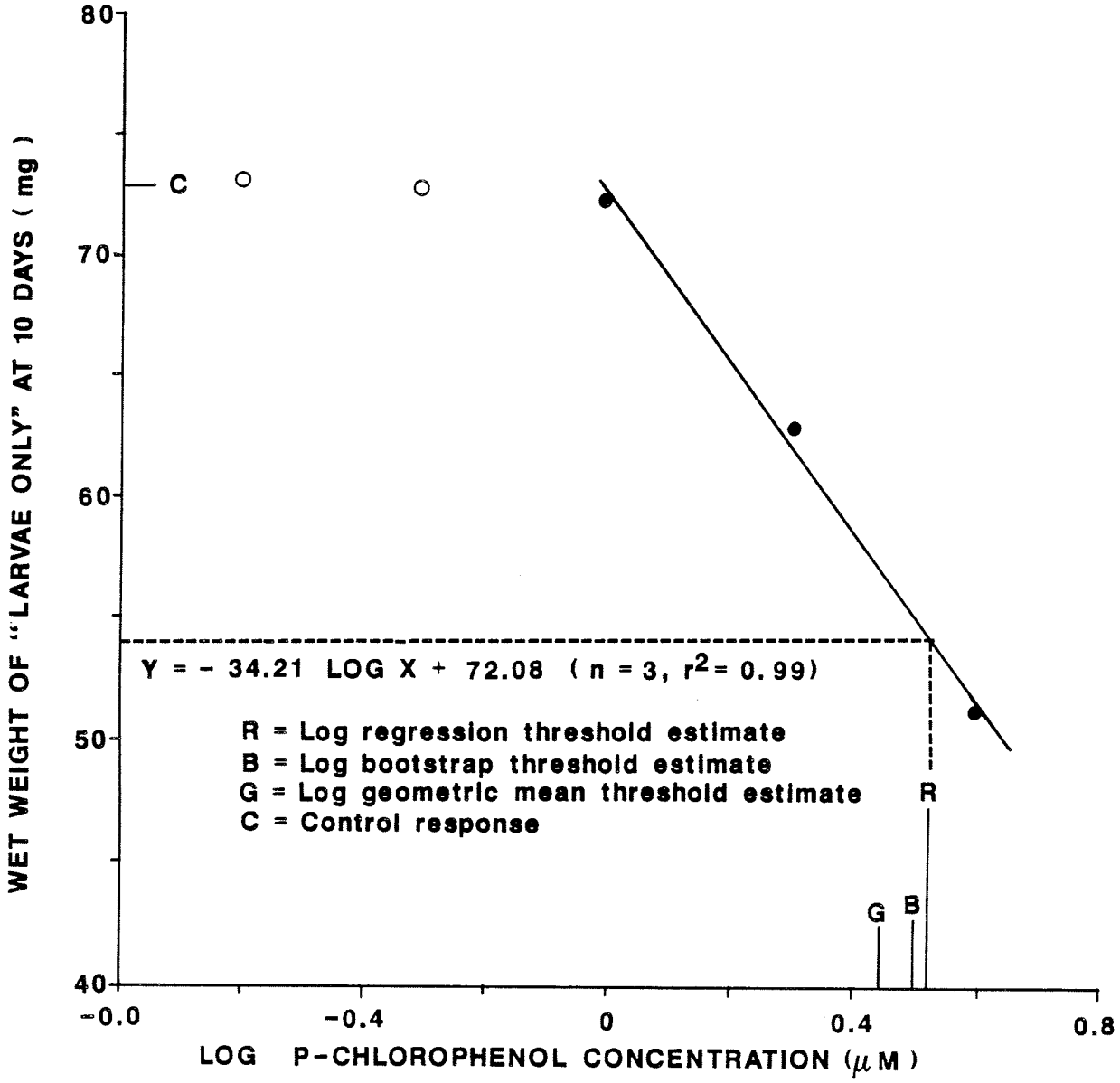

Figure 4. The effect of exposure to p-chlorophenol on the wet weight of "larvae only", 10 days after hatch. Threshold effect concentrations are shown for each statistical method of estimation. The regression is based on the filled circles.

TABLE 6

Mean responses of rainbow trout to p-chlorophenol exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
EGGS											
p-chlorophenol concentration (μM)	0.0	0.24	0.49	0.97	1.94	3.85					
% Mortality of eyed eggs	0.0	3.0	6.5	5.2	2.8	1.4	3.88	3.54	-		
% Hatch of eyed eggs	100.0	97.0	93.5	94.8	97.2	98.6	3.88	3.54	-		
% Died while hatching	0.0	0.6	1.6	2.4	1.2	0.8	1.38	1.26	-		
Degree days to hatch	364.8	358.9	369.5	365.7	361.3	367.7	4.36	3.98	2.47		
Wet weight of "larvae only" at hatch (mg)	30.6	31.3	26.3*	27.8*	27.7*	25.3*	0.60	0.54	48.80	1.72	6
Dry weight of "larvae only" at hatch (mg)	4.2	3.7*	3.5*	3.6*	3.6*	3.5*	0.14	0.13	7.90	0.41	10
Wet weight of yolk sac at hatch (mg)	50.8	48.6	49.8	50.7	51.1	50.0	1.77	1.61	0.77		
Dry weight of yolk sac at hatch (mg)	23.6	22.9	22.6	23.6	23.3	22.9	0.93	0.85	0.56		
Wet weight of larvae and yolk sac at hatch (mg)	81.4	79.9	76.1	78.5	78.8	75.3	2.60	2.37	2.07		
Dry weight of larvae and yolk sac at hatch (mg)	27.8	26.6	26.1	27.3	26.9	26.4	0.77	0.70	1.69		
% Moisture at hatch	86.2	88.3	86.9	87.1	87.0	86.2	0.78	0.71	2.67		
LARVAE											
p-chlorophenol concentration (μM)	0.0	0.24	0.49	0.97	1.94	3.85					
% Mortality of larvae	4.0	6.1	5.2	4.2	6.9	28.7*	7.88	7.19	4.39	22.86	23
% Deformed	13.2	15.1	17.1	14.0	13.0	16.7	3.80	3.56	0.20		
Degree days from hatch to swim-up	99.2	99.6	99.8	99.6	98.3	99.7	1.70	1.55	0.29		
Wet weight of "larvae only" at 10 days (mg)	72.6	74.3	73.3	72.1	63.1	51.5*	5.65	5.16	7.56	16.41	23
Dry weight of "larvae only" at 10 days (mg)	9.9	10.1	6.6	10.4	9.1	7.6	1.34	1.22	2.98		
Wet weight of yolk sac at 10 days (mg)	32.0	27.7	28.3	28.9	31.6	35.7	1.70	1.55	9.40	4.93	15
Dry weight of yolk sac at 10 days (mg)	15.9	13.8	13.9	14.2	15.5	17.4	0.84	0.76	8.40	2.42	15
Wet weight of larvae and yolk sac at 10 days (mg)	105.6	102.0	101.6	100.9	94.7	87.1*	5.00	4.56	4.88	14.50	14
Dry weight of larvae and yolk sac at 10 days (mg)	25.8	23.9	25.5	24.6	24.6	25.0	1.78	1.63	0.38		
Yolk sac conversion efficiency (wet)	2.28	2.13	2.26	2.05	1.84	1.92	0.45	0.41	0.44		
Yolk sac conversion efficiency (dry)	0.73	0.71	0.98	0.73	0.70	0.75	0.19	0.17	0.94		
% Moisture at 10 days	86.6	86.3	84.3	85.6	85.6	85.1	1.10	1.01	1.52		

TABLE 6 (continued)

Mean responses of rainbow trout to p-chlorophenol exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
FRY											
p-chlorophenol concentration (μM)	0.0	0.24	0.49	0.97	1.94	3.85					
% Mortality of fish	15.2	20.5	16.0	20.9	17.9	42.6*	9.06	8.27	3.73	26.30	26
Mortality per 1000 fish-days	3.4	5.0	3.9	5.2	4.7	13.1*	3.10	2.83	3.90	9.00	
Total number of fish-days	9653	9157	8642	8814	8568	7613	1202	1098	0.84		
Wet weight at week 4 post swim-up (mg)	491.1	470.5	512.2	496.6	480.7	304.7*	64.00	58.43	4.17	185.81	38
Dry weight at week 4 post swim-up (mg)	95.4	93.2	101.6	98.1	94.6	56.8*	13.00	12.14	4.56	38.60	40
% Moisture at week 4 post swim-up	80.6	80.2	80.2	80.4	80.3	81.7	0.55	0.50	3.63	1.59	2

S, standard deviation calculated from pooled error variance; s_d, standard difference between means used to calculate D value for Dunnett's test; F, F value from ANOVA; D, minimum significant difference from control in original units as calcultated by Dunnett's test; % diff., minimum significant percent difference from control.

* Treatment means significantly different from control by Dunnett's test.

"-" = no data.

TABLE 7

Mean responses of rainbow trout to 2,4-dichlorophenol exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
EGGS											
2,4-dichlorophenol concentration (μM)	0.0	0.61	1.10	1.96	3.44	6.14					
% Mortality of eyed eggs	0.6	0.3	0.9	1.1	2.3	2.2	1.10	0.90	1.70		
% Hatch of eyed eggs	99.4	99.7	99.1	98.9	97.7	97.8	1.13	0.92	1.68		
% Died while hatching	0.0	0.13	0.17	0.46	0.98	0.64	0.40	0.33	2.50		
Degree days to hatch	361.3	364.5	372.8	375.6	360.3	369.0	6.39	5.21	2.85		
Wet weight of "larvae only" at hatch (mg)	27.3	24.7	24.6	21.5*	21.0*	19.9*	1.92	1.57	6.35	4.88	18
Dry weight of "larvae only" at hatch (mg)	4.2	3.8	3.9	3.6	3.3	3.6	0.50	0.41	1.22		
Wet weight of yolk sac at hatch (mg)	48.0	49.4	49.8	51.8	54.4	44.8	3.96	3.23	2.05		
Dry weight of yolk sac at hatch (mg)	23.0	23.6	27.3	24.8	23.5	22.6	1.32	1.08	1.00		
Wet weight of larvae and yolk sac at hatch (mg)	75.2	24.1	74.4	72.7	75.5	64.7	4.54	3.70	2.43		
Dry weight of larvae and yolk sac at hatch (mg)	27.3	27.4	27.6	28.4	26.8	26.2	1.15	0.93	1.22		
% Moisture at hatch	84.8	84.7	84.1	83.0	84.2	81.6*	1.08	0.88	3.42	2.80	3
LARVAE											
2,4-dichlorophenol concentration (μM)	0.0	0.61	1.10	1.96	3.44	6.14					
% Mortality of larvae	3.7	4.8	30.5	74.3*	85.2*	89.6*	11.79	9.62	34.22	30.59	30
% Deformed	0.2	1.3	0.7	0.3	0.5	0.8	0.92	0.75	0.61		
Wet weight of "larvae only" at 10 days (mg)	57.8	47.3	46.7	30.8*	30.6*	28.0*	10.60	8.65	3.53	26.90	46
Dry weight of "larvae only" at 10 days (mg)	15.4	8.0	13.5	5.1*	5.6*	4.0*	2.99	2.44	3.34	7.56	49
Wet weight of yolk sac at 10 days (mg)	44.9	35.6*	38.5	42.9	41.2	41.1	9.06	7.39	0.39		
Dry weight of yolk sac at 10 days (mg)	16.5	17.9	22.5	20.8	20.0	15.7	3.97	3.24	1.33		
Wet weight of larvae and yolk sac at 10 days (mg)	107.2	82.8	85.2	73.7	71.8	86.4	16.55	13.50	1.34		
Dry weight of larvae and yolk sac at 10 days (mg)	31.9	25.9	36.0	25.9	25.6	22.8	6.05	4.94	1.98		
Yolk sac conversion efficiency (wet)	0.90	1.62	1.84	1.21	0.74	0.06	1.19	0.97	0.99		
Yolk sac conversion efficiency (dry)	1.75	0.74	1.62	0.38	1.02	0.22	1.32	1.08	0.69		
% Moisture at 10 days	75.0	83.0	65.8	83.4	82.2	85.0	14.47	11.81	0.78		

TABLE 7 (continued)

Mean responses of rainbow trout to 2,4-dichlorophenol exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
FRY											
2,4-dichlorophenol concentration (μM)	0.0	0.61	1.10	1.96	3.44	6.14					
% Mortality of fish	6.6	8.8	43.8*	86.2*	88.2*	89.7*	7.08	5.78	31.40	18.38	18
Mortality per 1000 fish-days	2.0	2.9	16.2	47.8*	54.8*	66.7*	7.44	6.07	43.07	19.30	
Total number of fish-days	6958	8029	6625	3974	3863	3667*	1230	1004	7.20	3191	46
Wet weight at week 4 post swim-up (mg)	704.0	427.3*	263.3*	212.0*	-	-	48.58	39.50	44.80	125.61	18
Dry weight at week 4 post swim-up (mg)	-	-	-	-	-	-					
% Moisture at week 4 post swim-up	-	-	-	-	-	-					

S, standard deviation calculated from pooled error variance; s_d, standard difference between means used to calculate D value for Dunnett's test; F, F value from ANOVA; D, minimum significant difference from control in original units as calculated by Dunnett's test; % diff., minimum significant percent difference from control.

* Treatment means significantly different from controls by Dunnett's test.

"-" = no data.

concentration, but there were no changes in percent moisture.

Fry mortality increased by 62% over controls at the highest test concentration, corresponding to an increase in mortality per 1000 fish-days of 3.71 to 43.4 (Table 8). No significant changes were recorded for the total number of fish-days.

Fry weights decreased significantly, but tests were performed on only four treatments, as the number of fry in each tank had dropped to less than 10 at the highest test concentration. Weight changes were monotonic and culminated in a 53% reduction relative to control (Figure 7). Slight increases in percent moisture for fry of about 2 to 3% relative to control were significant, but responses were not monotonic.

Pentachlorophenol

Results for PCP were obtained from Hodson and Blunt (1981). Although response means could not be compared statistically, the occurrence of exposure effects, as determined in the original publication by ANOVA ($p \leq 0.01$), are shown in Table 9.

A significant exposure effect was observed for larval weight at hatch. Weights decreased monotonically with a 27% reduction relative to control at the highest test concentration. Several symptoms of PCP toxicity to eggs are not discussed here and the reader is referred to the original work.

The ANOVA indicated significant increases in larval mortality per 1000 fish-days. At the highest test concentration, this corresponded to a change from control of 11.84 (Table 9). We assumed that this response was significant as all other test responses were similar to control. Weight of larvae at swim-up decreased monotonically and culminated in a 15% reduction relative to control. Yolk sac conversion efficiency decreased relative to control by 14% at the highest test concentration.

For fry, mortality per 1000 fish-days increased by up to 72.0 at the highest exposure level (Table 9). Only this value was different from the control value of 4.0. A significant concentration effect was observed for weights of fry at 4 weeks post swim-up. Weights decreased monotonically, culminating in a 72% decrease relative to the mean control value (Figure 8).

EFFECT THRESHOLD ESTIMATES

Regression estimation

Mortality parameters were less amenable to regression analysis than weight parameters, as mortality often increased only at the highest exposure level

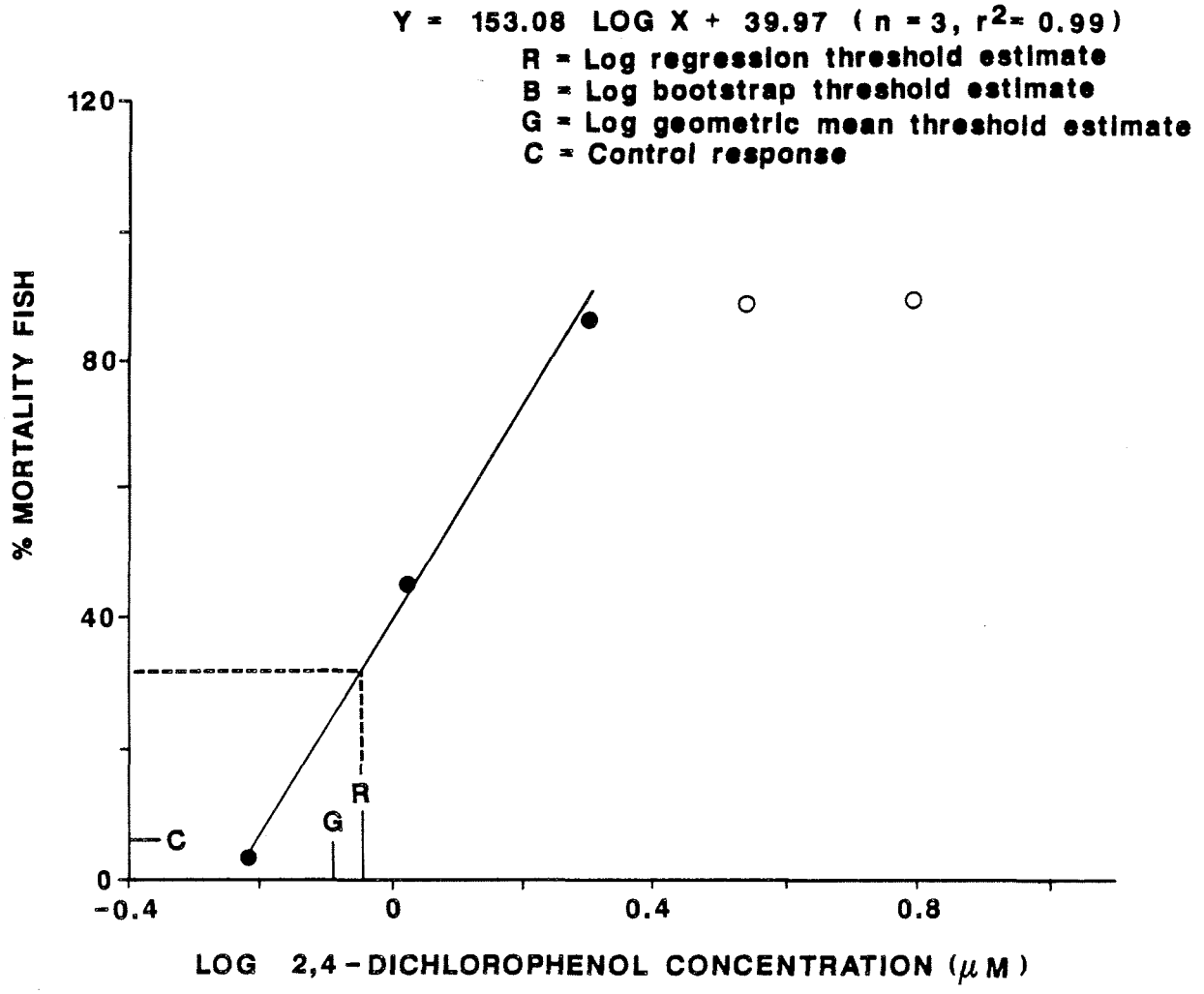

Figure 5. The effect of exposure to 2,4-dichlorophenol on the total mortality of rainbow trout four weeks after swim-up. Threshold effect concentrations are shown for each statistical method of estimation. The regression is based on the filled circles.

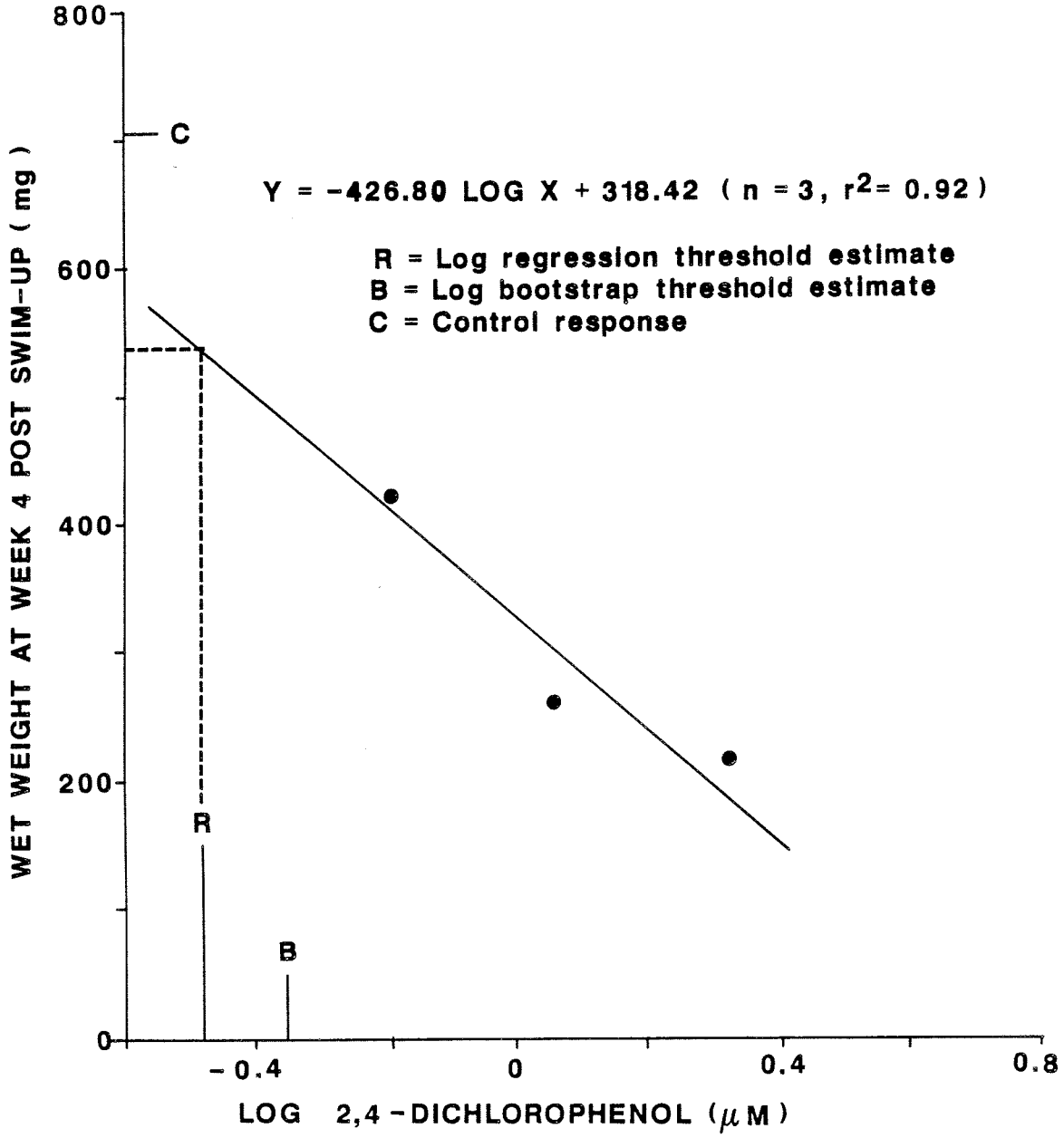

Figure 6. The effect of exposure to 2,4-dichlorophenol on the wet weight of fry four weeks after swim-up. Threshold effect concentrations are shown for each statistical method of estimation except the geometric mean.

(Table 9). The results of the regression analyses and the interpolated 25% minimum change thresholds are given in Table 10 for those parameters that met the criteria listed in the Materials and Methods. The correlation coefficients (r) in all cases were greater than 0.85, indicating that the regressions accounted for more than 72% ($r^2 > 0.72$) of the observed variation in responses.

Except for TCB, slopes (b) for the regressions were similar among experiments for wet weights of larvae at hatch and for wet weight at 10 days post-hatch (Table 10). Greater differences among experiments were observed for the slopes of wet weight of fry at 4 weeks post swim-up and for mortality parameters.

NOEC-LOEC geometric mean

The LOEC values for those parameters which were significantly different from control, and which differed by 25% or more, are listed in Table 11, along with the corresponding NOECs and geometric means. Reductions in larval and fry weight, and increases in mortality were the most consistent responses. While many changes were observed in percent moisture, the extent of changes were uniformly small and did not meet the criterion of a 25% change.

All chemicals except NP elicited a significant, 25% minimum reduction in weight for at least one of the two larval weight parameters (weight at hatch and at 10 days post-hatch) (Table 11). For NP, larval weight at hatch was reduced 37% relative to controls (Table 5), but this reduction was not significant due to large within-group variations. A 57% reduction would be needed to demonstrate a positive Dunnett's test.

All chemicals except PH caused a significant 25% minimum reduction in fry weight. Although fry weight declined by 27% in the PH test (Table 4), this change was not significant and a minimum percent difference from control of 70% would be required for a positive Dunnett's test. For other chemicals, the percent difference from control needed to demonstrate a significant change by Dunnett's test was 18% for larval weight and 20% for fry weight. In the PCP experiment, both larval weight at hatch and fry weight at 4 weeks post swim-up decreased relative to control by more than 25%. Significant treatment effects were demonstrated by ANOVA at a more conservative probability level of 0.01 (Table 9). Thus it is likely that the 25% minimum weight reductions observed for the PCP experiment represent significant reductions from control values.

LOECs based on dry weight measurements are also listed in Table 11 but are the same as those based on corresponding wet weights.

Weights of whole fish demonstrated a significant, 25% minimum reduction for the PH experiment

TABLE 8

Mean responses of rainbow trout to 1,2,4-trichlorobenzene exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
EGGS											
1,2,4-trichlorobenzene concentration (μM)	0.0	0.61	0.88	1.71	2.75	4.73					
% Mortality of eyed eggs	10.4	11.4	7.3	13.6	18.3	11.7	5.40	4.41	1.38		
% Hatch of eyed eggs	89.6	88.6	92.7	86.4	81.7	88.3	5.40	4.41	1.38		
% Died while hatching	7.3	7.9	5.6	10.5	16.8	10.7	4.90	4.00	1.96		
Degree days to hatch	346.7	343.9	346.4	346.0	350.2	347.9	5.01	4.09	0.71		
Wet weight of "larvae only" at hatch (mg)	27.1	25.3	23.9	25.5	26.5	23.2*	1.50	1.22	2.94	3.79	14
Dry weight of "larvae only" at hatch (mg)	3.5	3.4	3.2	3.3	3.6	3.0	0.23	0.19	2.49		
Wet weight of yolk sac at hatch (mg)	42.7	44.3	44.5	43.3	41.6	46.2*	1.30	1.06	4.23	3.30	8
Dry weight of yolk sac at hatch (mg)	19.8	20.3	20.7	20.1	19.3	21.3	0.62	0.50	4.00		
Wet weight of larvae and yolk sac at hatch (mg)	69.8	69.6	68.4	68.8	68.1	69.1	1.30	1.06	0.77		
Dry weight of larvae and yolk sac at hatch (mg)	23.4	23.7	24.0	23.4	22.8	24.3	0.53	0.43	2.94		
% Moisture at hatch	87.0	86.6	87.1	87.1	86.6	87.1	0.42	0.34	1.37		
LARVAE											
1,2,4-trichlorobenzene concentration (μM)	0.0	0.39	0.99	1.38	2.37	4.95					
% Mortality of larvae	7.2	9.6	8.8	6.6	5.8	66.7*	5.30	4.32	62.18	13.93	14
% Deformed	10.1	12.5	14.0	31.4*	12.5	28.6	8.31	6.78	3.74	21.01	21
Degree days from hatch to swim-up	103.4	104.0	105.0	103.8	102.9	105.6	1.21	0.99	2.16		
Wet weight of "larvae only" at 10 days (mg)	71.0	61.3*	59.7*	57.6*	55.6*	41.6*	2.40	1.96	48.19	6.10	9
Dry weight of "larvae only" at 10 days (mg)	10.1	8.7*	8.4*	8.1*	8.0*	5.5*	0.46	0.38	32.83	1.18	12
Wet weight of yolk sac at 10 days (mg)	24.8	27.1	27.3	28.6*	27.1	34.3*	1.20	0.98	21.57	305	12
Dry weight of yolk sac at 10 days (mg)	12.5	13.9	13.8	14.5*	13.4	15.9*	0.78	0.64	6.29	1.99	16
Wet weight of larvae and yolk sac at 10 days (mg)	95.8	88.4*	87.0*	86.1*	82.7*	75.9*	2.18	1.78	27.30	5.53	6
Dry weight of larvae and yolk sac at 10 days (mg)	22.7	22.6	22.2	22.6	21.4	21.4	0.69	0.56	2.37		
Yolk sac conversion efficiency (wet)	2.46	2.10	2.09	2.24	2.01	1.54*	0.26	0.21	3.99	0.65	26
Yolk sac conversion efficiency (dry)	0.90	0.84	0.75	0.89	0.76	0.45*	0.11	0.09	6.94	0.28	31
% Moisture at 10 days	85.7	85.7	85.9	85.9	85.6	86.9	0.43	0.35	3.35		

TABLE 8 (continued)

Mean responses of rainbow trout to 1,2,4-trichlorobenzene exposure and results of analysis of variance and Dunnett's test

	n										
	3	3	3	3	2	3	S	S_d	F	D	% diff.
FRY											
1,2,4-trichlorobenzene concentration (μM)	0.0	0.55	0.88	1.93	2.59	9.42					
% Mortality of fish	13.1	17.6	28.2	23.1	27.7	74.6*	9.17	7.48	16.70	23.26	23
Mortality per 1000 fish-days	3.71	5.0	9.0	6.7	7.8	43.4*	4.90	4.00	28.59	12.44	
Total number of fish-days	4375	3536	3383	3320	4055	2031	1405	1146	0.99		
Wet weight at week 4 post swim-up (mg)	539.0	586.0	420.0*	408.0*	252.0*	-	7.50	6.12	9.05	19.16	4
Dry weight at week 4 post swim-up (mg)	110.0	120.0	80.0	78.0	44.0*	-	16.2	13.22	10.21	41.38	38
% Moisture at week 4 post swim-up	79.5	79.6	81.2*	81.0	82.7*	-	0.61	0.50	13.65	1.56	2

S, standard deviation calculated from pooled error variance; S_d, standard difference between means used to calculate D value for Dunnett's test; F, F value from ANOVA; D, minimum significant difference from control in original units; % diff., minimum significant percent difference from control.

* Treatment means significantly different from control by Dunnett's test.

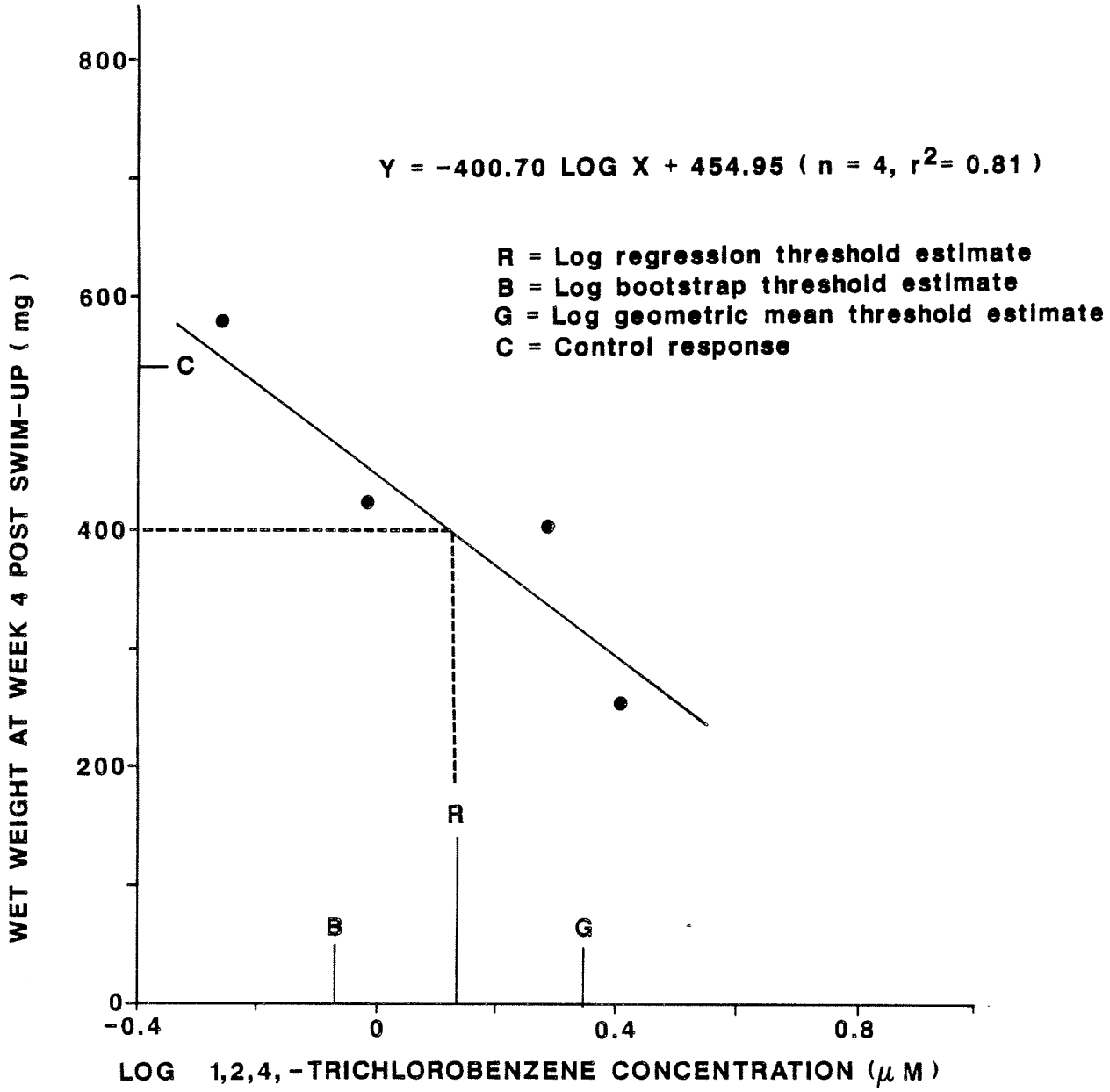

Figure 7. The effect of exposure to 1,2,4-trichlorobenzene on wet weight of rainbow trout four weeks after swim-up. Threshold effect concentrations are shown for each method of estimation.

TABLE 9

Mean responses of rainbow trout to pentachlorophenol exposure and results of analysis of variance

	n				CONCENTRATION* EFFECT
	2	2	2	2	
EGGS					
pentachlorophenol concentration (µM)	0.00	0.041	0.105	0.308	
% Mortality of eyed eggs	1.0	0.6	0.3	1.7	NO
Degree days to hatch	305.00	323.00	336.00	341.00	NO
Wet weight of "larvae only" at hatch (mg)	19.1	18.5	15.8	14.0*	YES
LARVAE					
pentachlorophenol concentration (µM)	0.00	0.060	0.094	0.330	
Mortality per 1000 fish-days	2.63	1.58	1.32	11.84	YES
Wet weight of "larvae only" at swim-up (mg)	81.8	81.0	77.7	69.5	YES
Yolk sac conversion efficiency (wet)	1.47	1.59	1.52	1.26	YES
FRY					
pentachlorophenol concentration (µM)	0.00	0.060	0.094	0.030	
Mortality per 1000 fish-days	4.00	2.96	2.94	72.00	YES
Wet weight at week 4 post swim-up (mg)	847.00	728.00	628.00*	234.00*	YES

* Treatment means differing from controls by 25% or more.

* Original factorial analysis of variance tested exposure, temperature, and exposure-temperature interaction effects at the 99% confidence level (Hodson and Blunt 1981).

only (Table 11). The corresponding LOEC value was about 4 times higher than the lowest LOEC for that experiment, which was based on reduction of larval weight at hatch.

Yolk weight conversion efficiency demonstrated a significant, 25% minimum reduction for the TCB experiment only (Table 11). The LOEC value was about 1.5 times that of the lowest LOEC for that chemical based on reduction of fry weight.

All chemicals, except NP, elicited a significant, 25% minimum increase in mortality (Table 11). For NP, percent mortality increased by only 12% relative to control mortality (Table 5), and a 19% increase would be required for a positive Dunnett's test.

Mortality per 1000 fish-days is cumulative, and is based on the number of fish in a tank, which decreases with time. Computer simulations were performed to estimate the increase in mortality per 1000 fish-days necessary to increase the percent mortality of fish by 25% over the period of fry growth. These simulations indicated that a 10-fold increase relative to control would be sufficient.

The LOECs for percent mortality of fish in the PH and DCP experiments were lower than those for mortality per 1000 fish-days by a factor of 1.8, or one test concentration level (Table 11); no LOEC was based on mortality per 1000 fish-days in the CP test. LOECs for hatch parameters were the same as those for mortality in the TCB experiment. No mortality parameters were significant in the NP study. In the PCP test, mortality per 1000 fish-days was the only mortality parameter available. From Table 9 it can be seen that fry mortality increases at the highest test concentration of PCP, with no change at lower exposure levels. Thus it is safe to conclude that the LOEC value for this parameter response was the highest PCP concentration.

The only other parameters to meet the criteria were those of yolk conversion efficiencies of TCB. The NOELs and LOECs were similar to those associated with weight changes (Table 11).

The lowest geometric mean estimates for reduction of larval or fry weights and for increased mortality are listed in Table 12. Weight reduction thresholds are lower than those for mortality except in the case of CP where they are the same. The geometric mean for weight reduction in the DCP experiment is given as a range since the NOEC was 0 uM (control). These threshold estimates are cruder than those estimated by regression analysis.

A comparison of the regression estimates of thresholds with the corresponding geometric means (Table 11) indicates a similar trend and magnitude in values. As

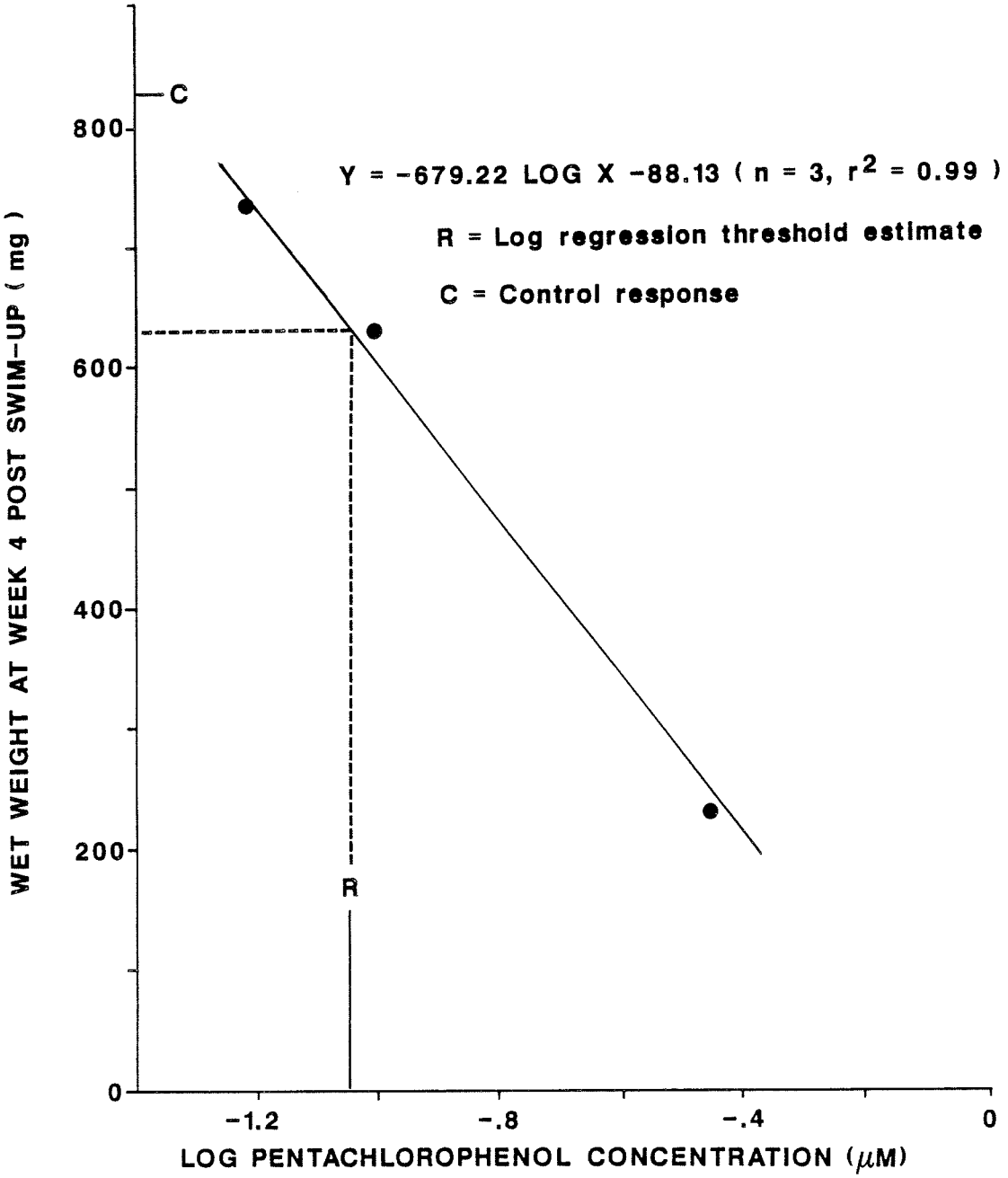

Figure 8. The effect of exposure to pentachlorophenol on the wet weight of rainbow trout at four weeks after swim-up. The threshold effect concentration was estimated from the regression.

TABLE 10

Linear regressions relating measurements to logarithm of concentration (y = a + b log X).
Thresholds are interpolated values associated with a 25% change relative to control measurements.

	n	a	b	r	Interpolated threshold (uM)
phenol					
Wet weight of "larvae only" at hatch	5	27.17	-5.79	-0.89	15.34
Dry weight of "larvae only" at hatch	4	4.05	-1.22	-0.99	9.70
Wet weight of "larvae only" at 10 days	5	74.94	-18.70	-0.91	19.60
Dry weight of "larvae only" at 10 days	5	10.36	-2.63	-0.88	16.88
Wet weight of larvae and yolk sac at 10 days	4	101.74	-18.31	-0.98	61.70
% mortality fish	3	-112.45	45.75	0.94	21.33
Mortality per 1000 fish days	3	-42.58	39.61	0.91	38.01
P-nitrophenol					
Wet weight at week 4 post swim-up	5	452.00	-158.06	-0.90	5.20
Dry weight at week 4 post swim-up	5	79.69	-31.47	-0.93	2.81
P-chlorophenol					
Wet weight of "larvae only" at 10 days	3	72.08	-34.21	-0.99	3.27
Wet weight at week 4 post swim-up	4	478.08	-213.34	-0.85	3.45
Dry weight at week 4 post swim-up	4	94.15	-46.08	-0.85	3.09
% mortality larvae	3	-1.60	40.20	0.91	4.80
2,4-dichlorophenol					
Wet weight of "larvae only" at hatch	5	23.86	-5.28	-0.95	4.32
Wet weight at week 4 post swim-up	5	43.20	-22.26	-0.91	0.98
Wet weight at week 4 post swim-up	3	318.42	-426.80	-0.96	0.32
% mortality larvae	4	29.84	114.35	0.98	1.00
% mortality fish	3	39.97	153.08	0.99	0.91
Mortality per 1000 fish-days	5	18.48	66.50	0.97	1.06

TABLE 10 (continued)

Linear regressions relating measurements to logarithm of concentration (y = a + b log X).
Thresholds are interpolated values associated with a 25% change relative to control measurements.

	n	a	b	r	Interpolated threshold (uM)
1,2,4-trichlorobenzene					
Wet weight of "larvae only" at 10 days	5	57.83	-16.76	-0.88	1.90
Dry weight of "larvae only" at 10 days	5	8.16	-2.64	-0.85	1.66
Wet weight at week 4 post swim-up	4	454.95	-400.70	-0.90	1.34
Dry weight at week 4 post swim-up	4	89.23	-90.99	-0.90	1.19
Yolk sac converison efficiency (wet)	3	2.44	-1.27	-0.99	2.97
Yolk sac conversion efficiency (dry)	3	1.02	-0.80	-0.99	2.70
pentachlorophenol					
Wet weight of "larvae only" at hatch	3	11.20	-5.22	-0.99	0.24
Wet weight at week 4 post swim-up	3	-88.13	-679.22	-0.99	0.09

n, number of observations; a, constant; b, regression coefficient or slope; r, correlation coefficient.

TABLE 11

Threshold concentrations associated with a 25% change relative to control values.

	NOEC (uM)	LOEC (uM)	Geometric mean (uM)	Bootstrap estimate with 95% confidence interval (uM)	Regression estimate (uM)
phenol					
*Wet weight of "larvae only" at hatch	8.46	16.00	11.63	14.58 (10.85 - 36.94)	15.34
*Dry weight of "larvae only" at hatch	8.46	16.00	11.63	9.09 (4.26 - 12.23)	9.70
*Wet weight of "larvae only" at 10 days	29.50	55.30	40.39	32.60 (13.91 - 39.43)	19.60
*Dry weight of "larvae only" at 10 days	29.50	55.30	40.39	31.86 (11.31 - 36.64)	16.88
*Wet weight of larvae and yolk sac at 10 days	29.50	55.30	40.39	53.70 (39.11 - 55.18)	61.70
*% Mortality fish	13.60	29.40	20.00	25.10 (23.01 - 26.95)	21.33
*Mortality per 1000 fish-days	29.40	42.30	35.26	---	38.01
p-nitrophenol					
*Wet weight at week 4 post swim-up	4.82	10.50	7.11	7.81 (1.89 - 14.43)	5.20
Dry weight at week 4 post swim-up	4.82	10.50	7.11	2.06 (1.42 - 11.98)	2.81
p-chlorophenol					
*Wet weight of "larvae only" at 10 days	1.94	3.88	2.74	3.21 (2.58 - 3.45)	3.27
Wet weight at week 4 post swim-up	1.94	3.88	2.74	3.15 (2.57 - 3.19)	3.45
Dry weight weight at week 4 post swim-up	1.94	3.88	2.74	3.03 (2.52 - 3.82)	3.09
*% Mortality larvae	1.94	3.88	2.74	3.79 (3.09 - 3.82)	4.80
% Mortality fish	1.94	3.88	2.74	---	-
2,4-dichlorophenol					
*Wet weight of "larvae only" at hatch	3.44	6.14	4.60	4.79 (1.37 - 5.96)	4.32
Wet weight at week 4 post swim-up	1.10	1.96	1.47	1.29 (0.52 - 1.79)	0.98
Wet weight at week 4 post swim-up	0.00	0.61	---	0.44 (0.32 - 0.62)	0.32
% Mortality larvae	1.10	1.96	1.47	---	1.00
*% Mortality fish	0.61	1.10	0.82	---	0.91
Mortality per 1000 fish-days	1.10	1.96	1.47	---	1.06

TABLE 11 (continued)

Threshold concentrations associated with a 25% change relative to control values.

	NOEC (uM)	LOEC (uM)	Geometric mean (uM)	Bootstrap estimate with 95% confidence interval (uM)	Regression estimate (uM)
1,2,4-trichlorobenzene					
*Wet weight of "larvae only" at 10 days	2.37	4.95	3.44	2.83 (2.06 - 3.29)	1.90
*Dry weight of "larvae only" at 10 days	2.37	4.95	3.44	2.81 (2.20 - 3.16)	1.66
*Wet weight at week 4 post swim-up	1.93	2.59	2.24	0.90 (0.80 - 2.00)	1.34
Dry weight at week 4 post swim-up	1.93	2.59	2.24	0.85 (0.77 - 1.91)	1.19
Yolk sac converison efficiency (wet)	2.37	4.95	3.44	3.29 (2.62 - 3.86)	2.97
Yolk sac conversion efficiency (dry)	2.37	4.95	3.44	3.13 (2.52 - 3.53)	2.70
*% Mortality larvae	2.37	4.95	3.44	---	-
*% Mortality fish	2.59	9.42	4.94	3.66 (2.39 - 4.55)	-
*% Mortality per 1000 fish-days	2.59	9.42	4.94	---	
**pentachlorophenol					
*Wet weight of "larvae only" at hatch	0.105	0.308	0.180	---	0.24
*Wet weight at week 4 post swim-up	0.041	0.105	0.066	---	0.09
*Mortality per 1000 fish days (fry)	0.094	0.330	0.176	---	-

* Responses exhibiting a 25% change relative to control which is statistically significant at the 95% confidence level.

** Dunnett's test not applicable to pentachlorophenol data. See text.

"-" = insufficient data for Bootstrap estimation.

in the case of thresholds estimated by the geometric mean, the lowest threshold estimated by regression is based on growth and not mortality. Linear regressions of weights vs log concentration are illustrated in Figures 2-8. Thresholds estimated by the regression method, geometric means, and the bootstrap method are shown for comparison.

Bootstrap estimation

Bootstrap estimates of the 25 percent effect concentration were calculated for those responses which exhibited a significant reduction relative to control values by 25% or greater. Percent mortality was transformed to percent survival (i.e. 100% - % mortality) but mortality per 1000 fish-days was not amenable to the bootstrap procedure. Mortality data for DCP, and all data for PCP were mean values, and "bootstrapping" could not be performed.

Bootstrap threshold estimates and their 95% confidence intervals were quite similar to their respective regression estimates (Table 11). This is understandable given the good correlation coefficients (r) for the regressions (Table 10) and the fact that bootstrap estimates are interpolated from an assumed linear function between any two adjacent means.

Summary

Regression estimates of the 25 percent effect threshold concentration for larval or fry weight reduction were available for all compounds tested. These thresholds were consistently lower (Table 11) than those derived from mortality responses. Threshold concentrations for NP, DCP, TCB and PCP were based on a 25% weight reduction for fry at 4 weeks post swim-up. The CP threshold was based on a 25% reduction in larval weight at 10 days post-hatch but the threshold associated with reduced fry weight was essentially the same as that for larval weight (3.27 vs 3.45 uM).

The threshold concentration for PH was based on a 25% decrease in larval weight at hatch. While fry weight was reduced by 27%, response variability was such that no significance was attached to this change. However, based on a 25% minimum change, without a statistical significance criterion, the NOEC and LOEC for fry weight reduction is 13.60 uM and 29.40 uM (Table 4), with a calculated geometric mean of 20.00 uM. This value is quite similar to the regression estimate of 15.34 uM, based on reduction of larval weight at hatch.

Therefore, threshold concentrations associated with weight changes of fry provide a consistent measure of chronic toxicity for the development of QSARs.

QSAR

The final chronic threshold concentrations for the six compounds are listed with 96 hour LC50s and the ratios of chronic to acute toxicity (Table 13). The rank order of chronic toxicity was similar to that for acute toxicity, except for DCP, which had a greater chronic toxicity than CP or TCB. The average value for the ratio of chronic to acute toxicity was 0.13, with a standard deviation of 0.066, or 50% of the average value. The ratio of chronic to acute toxicity for PH, TCB and PCP were consistent, but more variation was observed among the NP, CP and DCP ratios; they deviated from the average by about 30, 60 and 80% respectively.

QSARs for acute and chronic toxicity are given in Table 14 and appear parallel (Figure 9). Regressions (F-test) and slopes (t-test) for both QSARs were significant at the 0.05 probability level. The QSAR for acute toxicity accounted for more of the variability among LC50's (89%) than the chronic model for variability among thresholds (75%).

A t-test was performed to determine if the slopes of the two QSARs were homogenous. However, pooling of the sum of squares in a t-test for small sample sizes ($n \leq 30$) is only legitimate if the variance about the regression line is the same for each sample. Variance was assessed by calculating the ratio of the two variances (F-ratio) and determining if they were statistically different (F-test) at the 0.05 probability level. No significant difference was observed and the results of the t-test ($t = 2.67$, degrees of freedom = 8), indicated that the slopes for the acute and chronic QSARs were homogenous $p \leq 0.05$.

DISCUSSION

This experiment demonstrated that the chronic toxicity of a series of substituted phenols and benzenes increased with increasing octanol-water partition coefficients. Toxicity increased in the order phenol < p-nitrophenol < p-chlorophenol < 2,4-dichlorophenol < 1,2,4-trichlorobenzene < pentachlorophenol.

The comparison of control responses among the chronic tests provided perspective on treatment effects relative to "normal" variability. Mean control responses for weights of larvae and fry (Table 3) demonstrated increasing levels of variability for successive life stages. The standard deviations, calculated as a percent of the overall means, were: 8% for larval weight at hatch, 17% for larval weight at 10 days post-hatch and 28% for fry weight at 4 weeks post swim-up. The increased variability from hatch to 10-days post-hatch may indicate differences in yolk utilization, as larval weight increased by an average of 130% during this period.

TABLE 12

Comparison of threshold estimates based on larval or fry weight reduction and mortality increase.

COMPOUND	(NOEC-LOEC) GEOMETRIC MEAN (µM)		BOOTSTRAP ESTIMATE (µM)		REGRESSION ESTIMATE (µM)	
	WEIGHT	MORTALITY	WEIGHT	MORTALITY	WEIGHT	MORTALITY
phenol	11.63	20.00	14.58	25.10	15.34	21.33
P-nitrophenol	7.11	---	7.81	---	5.20	---
P-chlorophenol	2.74	2.74	3.21	3.79	3.27	4.80
2,4-dichlorophenol	0-0.61	0.82	0.44	---	0.32	0.91
1,2,4-trichlorobenzene	2.24	4.94	0.90	3.66	1.34	---
pentachlorophenol	0.07	0.18	---	---	0.09	---

Although yolk conversion efficiencies varied considerably, with a 20% standard deviation, they were not related to the percent increase in larval weights. Indeed, the highest control value for yolk conversion efficiency was associated with the lowest percent increase in larval weight. The standard deviation for weight of yolk at hatch was 9%, and at 10-days post-hatch, 33%; the increased error of yolk sac weights at 10-days post-hatch may be a function of their smaller size, since they were less easily dissected. Conversely, larger variation in the weights of larvae at 10-days post-hatch is likely the result of differences in yolk utilization, since larger larvae were more readily dissected and could be weighed with less relative error.

Fry weight exhibited the highest relative variation for measured growth in this study, which is characteristic for this stage of development. The fish are no longer self-sufficient, and differences in rations and aggressive behaviour of individuals due to density-dependent competition for food and space will emphasize differences in growth rates among individuals (Eaton and Farley, 1974). Variations in experimental conditions might also cause growth differences among experiments. However, post-hoc comparisons of growth responses were made within experiments, where conditions were more consistent.

Due to variability in growth, an average weight reduction for larvae and fry of 20% relative to control was required for a positive Dunnett's test ($p \leq 0.05$). Since a 25 percent weight reduction relative to control was chosen as the chronic toxicity end point, the threshold for toxicity was outside the bounds of normal variability.

There are many potential end points for estimating the chronic toxicity of chemicals to fish. In this study, mortality and simple growth, as measured by the weight of larvae or fry, proved to be the most consistent. Five of the six chemicals tested caused significant increases in mortality relative to control, and all of the chemicals decreased the weights of larvae and fry. Threshold concentrations based on reduced larval or fry weight were lower than those based on a 25% increase in mortality by an average factor of 0.48 (range: 0.25 to 0.67; calculated from Table 12).

For QSARs, weight reduction was chosen as the end point to ensure a complete and comparable set of chronic toxicity threshold concentrations.

However, only four of the final six chronic toxicity end points could be based on a single end-point: fry weight reduction. A 25% reduction in fry weight was not statistically significant in the case of PH and CP due to considerable within-group variation. Therefore, we chose weight reduction during the larval stage as an appropriate substitute. End points for PH and CP were based on

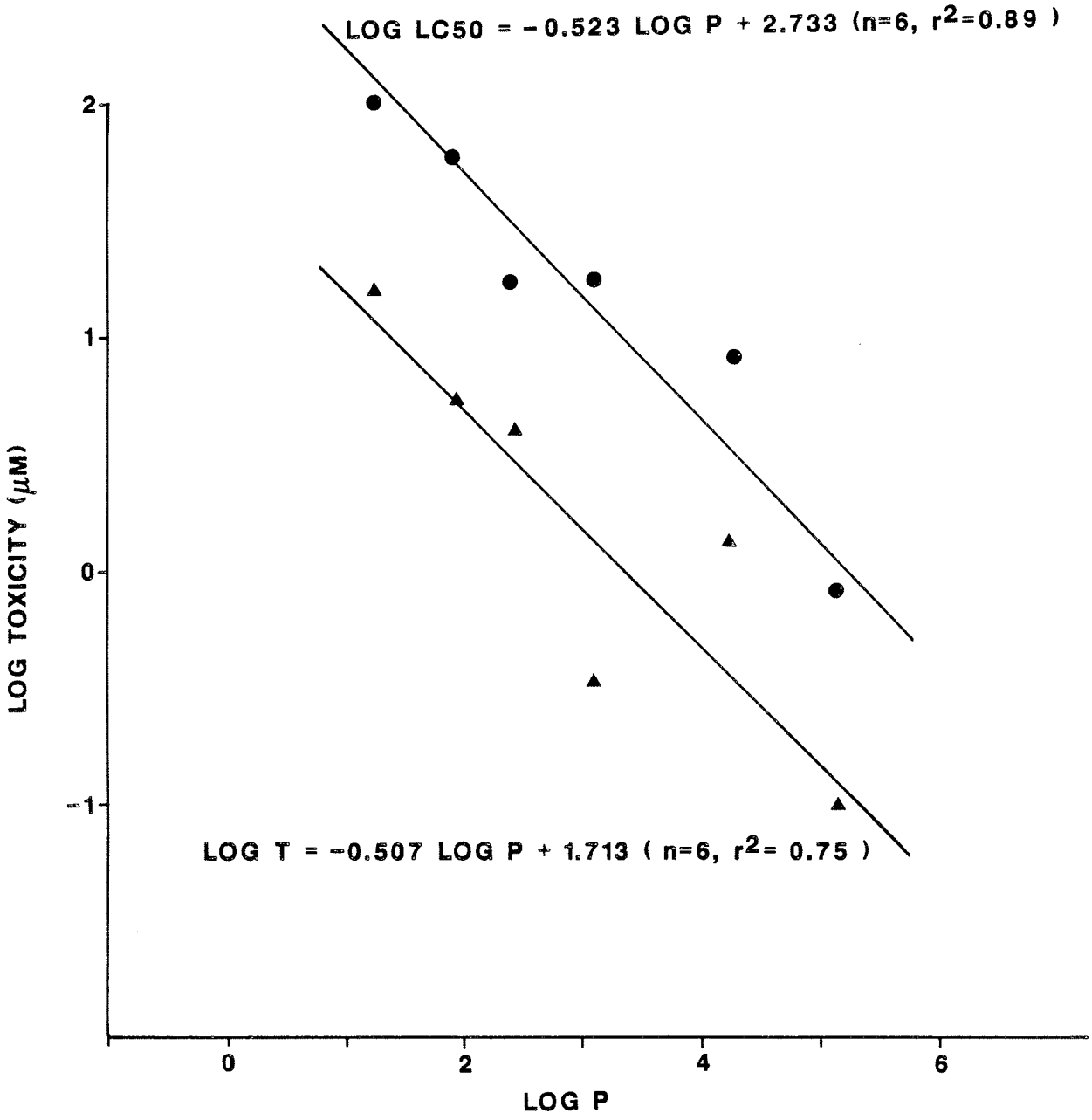

Figure 9. The relationship between octanol-water partition coefficient and measures of acute and chronic toxicity for phenol, p-nitrophenol, p-chlorophenol, 2,4-dichlorophenol, 1,2,4-trichlorobenzene and pentachlorophenol.

TABLE 13

Comparison of acute and chronic toxicity of test compounds to rainbow trout.

COMPOUND	LOG P	ACUTE TOXICITY (uM)	REGRESSION ESTIMATE OF CHRONIC TOXICITY (uM)	RATIO OF CHRONIC/ACUTE
phenol	1.49	103	15.34	0.15
p-nitrophenol	1.91	57	5.20	0.09
p-chlorophenol	2.42	14.8	3.27	0.22
2,4-dichlorophenol	3.08	16.0	0.32	0.02
1,2,4-trichlorobenzene	4.26	8.3	1.34	0.16
pentachlorophenol	5.12	0.6	0.09	0.15

TABLE 14

Relationship between log P and the logarithms of acute and chronic toxicity estimates (in uM) of the test chemicals*.

		n	S_b	T_b	F_r	S_y	r^2
Acute:	Log LC50 = - 0.523 log P + 2.733	6	0.09	-5.58	31.1	0.29	0.89
Chronic[b]:	Log T = - 0.507 log P + 1.713	6	0.14	-3.51	12.3	0.45	0.75

n, number of observation; S_b, standard deviation of the slope; t_b, t-test of significance of the slope; F_r, F-test of significance of the regression; S_y, standard error of the estimate; r^2, coefficient of determination.

* All slopes and regressions are statistically significant at the 0.05 probability level.

[b] Chronic toxicity thresholds (T) from regression estimates.

significant, 25 percent reductions in larval weight at hatch and at 10-days post-hatch respectively. Thresholds based on fry weight reduction would have been comparable to those based on larval weight decreases, but without the associated statistical significance. Weight reduction of larvae at hatch persisted to the fry stage for all six test chemicals. There was no recovery of weight gain by the end of the experiment as was observed in a study of the effects of dioxin on rainbow trout early life stages (Helder 1980).

The criterion of a 25% change is purely arbitrary and follows current practice in the United Sates (US EPA 1989). This criterion however, is not equivalent to environmental protection, nor is it suitable for all parameters. A good example is percent moisture. There were tests in which percent moisture changed significantly but deviated from control values by only 1 to 3 % (e.g. phenol, Table 4). Since percent moisture reflects both osmoregulation and the metabolism of lipid, small changes are important and may lead to other, larger effects (e.g. changes in percent mortality).

A more realistic approach to thresholds would be to link them to the natural variability of the parameter. From Table 2, it is evident that the range among experiments for percent moisture of larvae at hatch is 3.2%, while the range for percent hatch is 11.5% and percent mortality of eggs is 22.1%. Clearly these variables should not be treated identically.

From an ecological perspective, a 25% change in growth rate may have effects on biomass production that vary widely, depending on ecological conditions. The significance can only be assessed through estimates of population performance, perhaps through modelling. For percent mortality, a 25% increase for a period of 30 days of fry growth will lead to extinction of the population if the mortality rate persists.

Therefore, while a 25% change from control represents a convenient and common end-point, it is dangerous to use it for decisions on permissable chemical levels in water. Development of water quality criteria from these data will require a significant exercise of judgement, and not a simple mathematical calculation. Similarly, interpolating the toxicity of non-tested compounds from the QSAR for chronic toxicity, creates significant risk of under or over-estimation of potential effects.

The logarithm of chronic and acute toxicity was strongly correlated to the logarithm of the octanol/water partition coefficient (r^2 = 0.75 and 0.89 respectively), but the ratio of cases to independent variables was only 6 for this study (a bare minimum requirement is a ratio of 5) and large r^2 values may be artefacts. The regressions and slopes were

considered significant ($p \leq 0.05$), and the slopes were homogenous or equal ($p \leq 0.05$), indicating that chronic toxicity for these chemicals could be reasonably predicted on the basis of acute toxicity. Although the slopes appear identical (Figure 8), the greatest deviation about the two QSARs occurs at a log P of 3.08, for DCP. Since this partition coefficient is mid-range, variations in measured toxicity do not seriously affect the slopes of QSARs.

It is difficult to test the assumption that common modes of action are the basis for QSARs, i.e. that separate QSARs can be developed for each physical, reversible mechanism of toxic action (Arnold *et al. 1990*). The compounds tested in this study can be classified in three different groups: non-polar narcotics (TCB), polar narcotics (PH, NP, CP, DCP, PCP) and uncouplers of oxidative phosphorylation (PCP).

Narcosis by nonpolar compounds is thought to represent a reversible "slowing" of cytoplasmic activity in the cell due to chemical partitioning into the biophase (Schultz 1989).

Schultz *et al.* (1986), working with a heterogenous series of phenols, demonstrated two log P-dependent QSARs; one for polar narcotic chemicals and the other for "weak acid" uncouplers of oxidative phosphorylation. Uncouplers usually contain two nitro substituents or chlorine substituents in the 4 or 5 position, as is the case for PCP. PCP uncouples oxidative phosphorylation (Weinbach 1957) so that less adenosine triphospate (ATP) is available for normal metabolic processes. To compensate, more energy is directed to ATP production which increases basal metabolism and reduces energy available for growth.

This reduced growth efficiency should be evident in reduced yolk conversion efficiencies. PCP and TCB were the only compounds that caused statistically significant reductions in yolk conversion efficiency. Although decreases were noted for the other compounds, variability was too high to distinguish significant effects.

Therefore, the QSARs presented here represent a mixed model, in that several mechanisms of toxicity may be acting. Nevertheless, strong relationships were observed. Since log P mimics the partitioning of chemicals from water into lipid, QSARs based on log P suggest that, for these compounds, the kinetics of chemical accumulation are more important determinants of toxicity than specific mechanisms. Nevertheless, specific mechanisms may explain some of the variability about the regressions.

Ionization of the phenolics in water may also contribute to variability about the QSAR regression. Saarikoski and Viluksela (1981) showed that ionization of phenols and toxicity varied with pH, presumably because

pH controls the relative proportion of the more polar ion of each phenol, which is taken up at a lower rate the non-polar parent compound.

We have assumed that the expressed toxicity of these chemicals was a function of a steady-state exposure, where levels of contaminants at the site of toxic action are virtually constant. While this may be the case for the phenols, which rapidly reach equilibrium in tissue (<< 4 days, McCarty 1990), Oliver and Niimi (1983) demonstrated that the rate of accumulation of chlorobenzenes by trout decreased with an increasing degree of chlorination. For TCB, equilibrium was reached in 20 days, well within the 85-day span of this experiment.

The average ratio of chronic to acute (96 hr LC50) toxicities was 0.13. A second estimate of this ratio may be calculated from the ratios of the antilogs of the intercepts of the two QSARs (0.095). However, individual ratios varied considerably and DCP (ratio = 0.02) differed most from the overall mean. The DCP threshold concentration may represent an extreme case since its chronic toxicity is much greater than might be expected on the basis of its acute toxicity. The chronic threshold concentration for DCP was a low 0.32 µM, based on growth, but was consistent with the threshold concentration of 0.91 µM for chronic mortality. Three- to five-fold differences among laboratories in the threshold concentrations derived for the same species and for the same test chemical are typical (Woltering 1984). Nevertheless, the ratio can only be considered in error if we accept the assumption that all test chemicals have the same modes of acute and chronic toxic action, and hence the same ratios. We have no evidence with which to test this assumption.

The average ratio and the intercept of the chronic toxicity QSAR are also not fixed, i.e. they will vary according to which response is chosen as the basis for the QSAR and what the criterion is for a significant response.

The data published here were first summarized as a preliminary QSAR analysis by McCarty *et al.* (1985). Their QSAR for the chronic toxicity of chlorophenolics was not parallel to their QSAR for acute toxicity, in contrast to our analysis. The differences in the QSARs are a function of the way the data were analyzed:

1. The data were scrutinized for errors much more carefully in this study;

2. We estimated thresholds only for those parameters that changed from control by more than 25 %;

3. McCarty *et al.* (1985) used Duncan's New Multiple Range Test to identify treatment means that were different from control. This test is less reliable than Dunnett's test due to indeterminant experimental error (Day and Quinn 1989);

4. The chronic threshold concentrations were estimated by McCarty *et al.* (1985) using the NOEC-LOEC approach; we used regression analyses;

5. McCarty *et al.* (1985) used several end-points to calculation each threshold; we consistently used growth of fry to estimate thresholds;

6. The QSAR in this analysis included NP and TCB where as McCarty *et al.* (1985) included only chlorophenolics.

In summary, a QSAR was described for the chronic toxicity of organic chemicals to fish. This QSAR was parallel to that for acute toxicity. The difference between their intercepts was about one log unit, indicating an average ratio of chronic to acute toxicity of about 0.10.

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