Use of Sodium Pentachlorophenate and Dehydroabietic Acid as Reference Toxicants for Salmonid Bioassays

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USE OF SODIUM PENTACHLOROPHENATE AND DEHYDROABIETIC ACID AS REFERENCE TOXICANTS FOR SALMONID BIOASSAYS - AN INTERLABORATORY COOPERATIVE STUDY

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ABSTRACT

Davis, J.C. and Hoos, R.A.W. 1974. Use of sodium pentachlorophenate and dehydroabietic acid as reference toxicants for salmonid bioassays - an interlaboratory cooperative study. Fisheries and Marine Service Tech. Rept. No. 464, 11 p + 2 Appendices.

Results of an inter-laboratory bioassay standardization exercise involving 7 independent fish bioassay laboratories in British Columbia are presented. Toxicity of standard solutions of sodium pentachlorophenate and dehydroabietic acid was determined in freshwater static bioassays with underyearling rainbow trout (*Salmo gairdneri*), coho (*Oncorhynchus kisutch*) and sockeye (*Oncorhynchus nerka*) salmon. Reported LC50's for the three species ranged from 37 - 130 ppb for sodium pentachlorophenate and 1.03 - 2.14 ppm for dehydroabietic acid. In general, test results for individual species and toxicants were fairly consistent and major disparities could be explained in some instances by variations in physical and chemical characteristics of the bioassay such as water temperature, hardness or pH. Fish size and condition factor appeared to have little effect on apparent toxicity over the ranges tested.

The usefulness of reference toxicants for standardizing bioassays is emphasized and suggestions are made for improved test procedures and increasing accuracy of results. Guidelines for toxicity tests should include the use of reference toxicants as a means of standardizing bioassay results.

RESUME

L'article présente les résultats d'un travail de normalisation des dosages biologiques auquel ont participé sept laboratoires indépendants de la Columbie-Britannique, spécialisés dans les épreuves de contrôle biologique duspoisson. La toxicité de solutions standard de pentachloro phénate de sodium et d'acide déshydroabiétique a été determinée par dosages biologiques statiques effectués en eau douce sur des truites arc-en-ciel (Salmo gairdneri) des saumons cohos (Oncorhynchus kisutch) et des saumons rouges (Oncorhynchus nerka), tous âgés de moins d'un an. Les DL50 trouvées pour les trois espèces ont varié de 37 à 130 p.p.b., pour le pentachlorophénate de sodium, et de 1.03 à 2.14 p.p.m., pour l'acide déshydroabiétique. Les résultats des tests individuels pour les espèces et les toxiques ont généralement été d'une bonne compatibilité, et les principales différences peuvent être en partie expliquées par des variations des caractéristiques physiques et chimiques du dosage biologique telles que la température, la dureté et le pH de l'eau. La taille et l'état des poissons ont semblé avoir eu peu d'effet sur la toxicité apparente, dans les intervalles étudiées.

On souligne l'utilité de toxiques de référence pour la normalisation des dosages biologiques et l'on fait des suggestions pour le perfectionnement des méthodes et l'augmentation de la précision des résultats. Les lignes directrices des tests de toxicité devraient comprendre l'utilisation de toxiques de référence comme moyen de normalisation des résultats des dosages biologiques.

INTRODUCTION

This study was a bioassay standardization exercise involving seven laboratories, both government and private, in the lower mainland/coastal British Columbia area. Each test laboratory was provided with identical reference toxicant materials and a comprehensive set of guidelines for conducting standard 96hr static bioassays so that results obtained by various laboratories could be compared.

Considerable interest exists in methods of standardization for bioassays. Recently, federal legislation was promulgated for regulating the toxicity of pulp and paper wastes and petroleum refinery wastes and specific legislation for other toxicants is forthcoming or pending. These regulations propose effluent toxicity standards to be applied uniformly nationwide for each type of pollutant. The inclusion of reference toxicant tests, along with effluent toxicity tests, could improve the quality of the test procedure for comparative purposes. So far, use of reference toxicants for bioassay standardization appears rare and has only been applied to testing oil dispersants (LaRoche et al, 1970)[1] and pesticides (Marking, 1966).

Methods

Details of the location and water characteristics for each laboratory are given in Table 1.

Samples of two reference toxicants were distributed to each laboratory within a 24 hour period accompanied by a detailed set of

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[1] EPS Report 1 EE 73-1

Table 1

A list of the laboratories involved in the standardization exercise, their water source and its pre-bioassay treatment.

Table 1

Laboratory	Address	Water Source	Water Treatment
Environmental Protection Service	4160 Marine Dr. West Vancouver	Vancouver City Water Cleveland Dam	none
B.C. Research	3650 Westbrook Cres. Vancouver	Vancouver City Water Cleveland Dam	dechlorinated
Fisheries Research and Development	4160 Marine Dr. West Vancouver	Well Water - from site	aeration
Pollutech Pollution Advisory Services Ltd.	104 Charles St. North Vancouver	North Vancouver City Water - Seymour River	dechlorinated
Beak Consultants Ltd.	124 S.E. Marine Dr. Vancouver	Vancouver City Water Cleveland Dam	dechlorinated
International Pacific Salmon Fisheries Commission	Sweltzer Creek Station Cultus Lake, B.C.	Cultus Lake Water	none
Province of B.C. Pollution Control Branch	Fish & Wildlife Lab. 1019 Wharf Street Victoria	Victoria City Water Sooke Lake	1 megohm deionized

instructions (Appendix 1). One stock solution containing 14,418 ppb sodium pentachlorophenate (NaPcP) was prepared from Reagent Grade, 2, 3, 4, 5, 6 - pentachlorophenol (MW 266.36) by addition to NaOH, according to the procedure of Alderdice (1963). A second stock solution of 6,667 ppm dehydroabietic acid (DHAA), prepared from heat disproportionated pine resin (Hercules - 731 D)[2] according to the procedure of Halbrook and Lawrence (1966),was also provided. Dehydroabietic acid is a resin acid derived from wood and is a major toxicant present in wood processing wastes. Sodium pentachlorophenate is a widely used wood preservative.

The bioassay instructions adhered as closely as was practical to federal bioassay guidelines for pulp and paper wastes. Solution pH was adjusted prior to the test to 7.0 ± 0.1 using NaOH and HCl and was monitored throughout the test along with temperature and dissolved oxygen. Temperature and hardness were set equivalent to fish holding conditions for the test. Consultation with all laboratories locally indicated that it was possible to conduct the tests at 10 ± 2°C. These test conditions and procedures were based upon best available knowledge and practicality after joint discussion with the participants.

Replicate 96hr static bioassays in freshwater were conducted using five toxicant concentrations and two controls run simultaneously. Test fish were 1 - 3 gram rainbow trout (*Salmo gairdneri*), coho salmon (*Oncorhynchus kisutch*) or sockeye salmon (*Oncorhynchus nerka*) according to the stock availability at each laboratory. No attempt was made to standardize the source of test fish. Fish loading density in bioassays was specified as 0.5 g/l over the 4 day period and 10 fish were to be used/tank unless loading density requirements necessitated

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[2]Hercules Inc., Pine & Paper Chemicals Dept. Wilmington, Delaware, U.S.A.

Table 2:

Results of static bioassays and summary of test conditions reported by the various laboratories coded in a random fashion. LC50's were calculated in two ways, as a log-probit estimate or as an estimate resulting from a nomographic analysis yielding 95% confidence limits for the LC50 (upper and lower limits in brackets). The slope function, S, for the nomographic analysis is given. Fish condition factor, C.F., was calculated using the mean fish weight and length given in the table for each test.

Table 2

Lab. Code	LC50 Log-Probit Estimate	LC50 Nomographic Calc.+95% Con. Limits	S	loading density g/l	$\bar{x}$ initial temp-°C	$\bar{x}$ initial pH	$\bar{x}$ hard-ness ppm	$\bar{x}$ fish wt. g	$\bar{x}$ fish leng. cm	C.F. Wt/L^3
		rainbow trout - ppb sodium pentachlorophenate								
E	92	98(87.5, 109.8)	1.136	0.52	12.0	6.96	51.5	2.84	5.98	1.33
D	96	96(90.1, 102.2)	1.109	0.42	11.54	7.0	47.0	0.87	4.28	1.11
C	50	50(29.8, 84.0)	1.99	0.48	11.0±0.1	7.0±0.1	5-6	1.90	5.80	0.97
B	100	106(88.5, 127.0)	1.331	≈0.50	11.5	7.02	5.0	1.52	5.24	1.06
A	48	47(32.9, 67.2)	1.732	≈0.50	10.04	5.7	4.0	1.39	4.84	1.23
F	75	--	--	0.58	10.0±1.0	7.0±0.1	10.0	1.31	--	--
		rainbow trout - ppm dehydroabietic acid								
E	1.03	1.06(0.99, 1.14)	1.116	0.54	10.94	6.98	23.0	3.33	6.32	1.31
D	1.05	--*	--	0.57	12.25	6.97	47.0	1.26	--	--
C	1.22	--	--	0.48	11.0±0.1	7.0±0.1	5-6	1.90	5.80	0.97
B	1.74	1.85(1.62, 2.11)	1.237	≈0.50	11.5	6.92	5.0	1.40	5.32	0.93
A	1.18	1.20(1.03, 1.39)	1.118	≈0.50	9.3	9.0	4.0	1.67	4.96	1.37
F	1.25	--	--	0.57	10±1.0	7.0±0.1	10.0	1.29	--	--
		coho salmon - ppb sodium pentachlorophenate								
E	96	92(79.3, 106.7)	1.12	0.76	10.25	7.01	14.7	4.61	7.40	1.14
C	37	31.8(24.9, 40.6)	1.317	0.52	11.0±0.1	7.01±0.1	5-6	2.14	5.80	1.10
		coho salmon - ppm dehydroabietic acid								
E	1.38	--	--	0.69	10.62	6.97	7.0	4.18	6.24	1.72
C	1.76	1.75(1.56, 1.96)	1.134	0.52	11.0±0.1	7.1±0.1	5-6	2.14	5.80	1.10

* Data insufficient for calculation of confidence limits

Table 2 (cont'd)

Lab. Code	LC50 Log-Probit Estimate	LC50 Nomographic Calc.+95% Con. Limits	S	loading density g/l	$\bar{x}$ initial temp-°C	$\bar{x}$ initial pH	$\bar{x}$ hard-ness ppm	$\bar{x}$ fish wt. g	$\bar{x}$ fish leng. cm	C.F. Wt/L^3
		sockeye salmon - ppm dehydroabietic acid								
D	1.38	--	--	0.46	11.56	7.07	47.0	1.70	--	--
G	2.14	2.23(2.03, 2.45)	1.114	0.45	7.5	7.7	85.0	1.38	4.92	1.16
		sockeye salmon - ppb sodium pentachlorophenate								
D	50	50(40.3, 62.0)	1.281	0.56	12.5	7.19	47.0	0.68	--	--
G	130	130(119, 142)	1.096	0.46	7.5	7.70	85.0	1.38	5.05	1.07

more tanks with less fish/tank. Tests were done in 30 liters of aerated solution (> 90% O_2 satn.) in covered tanks.

Bioassays were observed at 30 minutes, 2, 6, 24, 72 and 96 hours after the beginning of the test and mortality was recorded as time to cessation of all movement by individual fish. Cumulative percentage mortality at each toxicant concentration was plotted on log-probit paper to estimate the time of 50% mortality (LT50). The incipient 96 hour LC50 was estimated in two ways. The first method involved plotting percentage mortality at 96 hours for each concentration on log-probit paper and reading the estimated concentration lethal to 50% of the fish from the graph (Sprague, 1969). If the data were sufficient, a second determination of 96 hour LC50 was carried out which involved fitting a line to log-probit data, expressed as concentration vs % dead at 96 hours, by the nomographic procedures of Litchfield and Wilcoxon (1949). This latter procedure estimates the line of best fit by minimizing its Chi^2 value and allows calculation of 95% confidence limits for the LC50. Slope function, or S, is also calculated so that other workers can reproduce the line, if desired. The LC50's produced by the above procedures are defined as the concentration lethal to 50% of the fish in 96 hours under the conditions of the test. Appendix 2 gives details of the analytical procedure.

Results

Results of the bioassays with both reference toxicants, as presented by the seven laboratories, using the 3 species, are summarized in Table 2. The laboratories are not identified but are

coded in a random fashion. Pertinent data including fish loading density, temperature, pH, water hardness, fish weight and fish length are reported. Fish condition factor was calculated from wt/leng.[3] where:

wt = weight in g, leng. = length in cm (Lagler, 1956).

Two estimates of LC50 appear in Table 2. The nomographic LC50, complete with confidence limits, allows comparison of different LC50's (LC50's can be considered different if their respective 95% confidence limits do not overlap - Sprague, personal communication). Clearly, there are both similarities and disparities between LC50's determined for the various laboratories.

Discussion

The results and pertinent information provided by each laboratory in Table 2 indicate that LC50's, as compared by examining the confidence limits, are moderately comparable. For example, results for rainbow trout in sodium pentachlorophenate indicated that LC50's obtained by labs. E, D and B were comparable. Similarly, those for labs. C & A were comparable but significantly lower than those for labs. E, D and B. The apparently greater sensitivity of fish used by labs C and A could be due, in part, to the low initial solution pH present for lab. A or to some factor not evident in the results such as general fish health, state of activity etc. Alderdice (1963) reported that the dissociation ratio of sodium pentachlorophenate to ~~sodium~~ pentachlorophenol is pH dependent. At pH's above 7.4 it is 99% ionized. Progressively more of the more toxic

phenolic moiety is present as pH drops below 7.4. Similarly, the differences in sockeye underyearling LC50's in dehydroabietic acid may be related to the disparity of water hardness and test temperature reported by the two laboratories testing that substance.

It should be emphasized that LC50's reported for dehydroabietic acid may be somewhat high as they represent theoretical concentrations in solution. G.M. Kruzynski (personal communication) found that in continuous flow bioassays an average of approximately 89% of dehydroabietic acid was actually present in solution as determined by gas chromatography of water samples in the theoretical concentration range 2.0 - 0.63 ppm. He also found that dehydroabietic acid concentration in static bioassays declines considerably with time. For this reason, this substance may be a poor choice as a reference toxicant unless gas chromatographic procedures are employed to accurately determine the actual solution concentration available during the bioassay.

No apparent trend was discernable between fish size or condition factor for the groups used nor was there a large difference in species sensitivity. Lack of a condition factor effect is in general agreement with the findings of Davis & Mason(1973) who reported that a condition factor range of 0.8 - 1.4 g/cm^3 did not affect toxicity test results with bleached kraft pulp mill effluent.

An observation made by many of the participants was that the data were weakened considerably by a lack of frequent observations, particularly in the early part (first 24 hours) of the bioassay. The choice of infrequent observations was partly based on economic

and personnel considerations of the private companies participating.

Sprague (1973) suggested the following convenient set of observation times:

Initial hour	Minutes: 15, 30, 60
First day and a half	Hours: 2, 4, 8, 14±2, 24, 33±3
2nd day, etc.	Days: 2, 3, 4 and daily beyond this

He points out that if a test is begun in mid-morning all observations can be made during normal working hours, with only one observation needed late in the evening of the first day.

Data quality was also influenced by the choice of test concentrations used for bioassays. For example, no fish would die in 96 hours in a given concentration while at the next concentration mortality could be at, or close to, 100% in 96 hours. With this sort of data, LC50 can only be crudely estimated by log-probit methods owing to the paucity of points available for plotting. In addition, use of the nomographic procedure of Litchfield and Wilcoxon (1949), is not possible as a plot of partial mortality (probit scale) vs. concentration (log scale) is required.

The data could therefore have been strengthened by inclusion of more replicates of intermediate toxicant concentrations, particularly those concentrations close to the LC50 value. It seems wise to suggest that at least 3 test concentrations be used between the concentration where little or no death occurs, and that where all or most of the fish die within 24 - 48 hours. Standard Methods (1971) gives useful tables for preparing dilutions of test solutions.

To our knowledge, little work has been done using reference toxicants as a means of standardizing bioassay procedures. Marking (1966) evaluated p, p′ -DDT as a reference toxicant for bioassay use. LaRoche et al, (1970) suggest "the use of a reference toxicant is desirable as a link between the findings of different investigators, as an internal standard to compare the relative toxicities of substances, and as a measure of condition of test organisms". The Canadian Environmental Protection Service's Report (EPS 1-EE-73-1, 1973) recommends use of reagent grade dodecyl sodium sulphate (also known as DSS or sodium lauryl sulfate) as being "rapid, nonselective and consistent in its toxicity to aquatic animals". That report requires that the median lethal DSS concentration be determined each time an oil dispersant bioassay is conducted. The report suggests that the median lethal DSS concentration may be in the general range of 1 - 10 mg/l. Apparently one problem associated with the use of DSS is its tendency to foam when used in continuous-flow bioassays (D.G. Alexander, personal communication). Another problem with DSS may be varying purity as received from the manufacturer.

Alderdice (1963) selected sodium pentachlorophenate, as being a suitable "toxic stressor" for fish after screening thirty five chemicals of considerable toxicity to fish. His selection was based on the following properties of sodium pentachlorophenate:

"high solubility in water

virtually complete ionization in solution at pH 7.0 or higher

stability in solution at pH 7.0 - 8.5

availability as a dry solid of high purity
facility of quantitative determination
toxicity to fish (in low concentrations)"

These criteria would seem to be excellent guidelines for the selection of other reference toxicants although the necessity for complete ionization is not applicable to toxic organics such as pulp mill waste constituents. Ideally, a group of substances should be available, selected from a list of major toxicants such as pesticides, detergents, heavy metals, wood-processing effluent, petrochemical wastes and other pollutants. Equally useful might be some stressor such as lethal temperature or salinity. Howard (1973) used lethal temperature as a stressor to evaluate the response of fish to kraft pulp mill waste, copper, zinc and phenol.

It should be emphasized that bioassay results can be affected by many factors including fish loading density, fish size and age, photoperiod, fish handling and exposure to visual stress, temperature, acclimation, water hardness and pH, aeration and others. Great care must be exercised in comparing bioassay results to insure that a meaningful comparison is possible. Guidelines and pertinent references for conducting bioassays are found in Sprague (1969), Standard Methods (1971) and Davis & Mason (1973).

In summary, the use of reference toxicants, such as sodium pentachlorophenate and dehydroabietic acid, yields useful information on the comparability of fish toxicity tests by independent laboratories. Fairly consistent results can be obtained provided a detailed set of guidelines is provided and criteria such as fish loading density, acclimation, test temperature, test tank size, statistical

procedures, frequency of observation, water pH and volume of test solution are specified. Reference toxicants provide a means of detecting large differences in sensitivity of fish stocks to toxicants that may be the result of the state of health of the stock or some variation in one of the above bioassay conditions. It is probably naive, however, to expect that any 2 stocks or bioassay tests will yield identical results owing to the tremendous scope of biological and environmental variation. Indeed the study of Marking (1966), involving toxicity of p, p/-DDT to 19 species of freshwater fish, failed to demonstrate consistency of toxicity for many of these species. The usefulness of reference toxicants therefore, may be confined to detecting large differences in sensitivity of test fish, which are likely to have a serious effect on bioassay results. Guidelines for toxicity tests should include the use of reference toxicants as a means of standardizing bioassay results.

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APPENDIX I

Bioassay Instructions Given to Each Lab.

REFERENCE TOXICANTS AND CONCENTRATIONS TO BE TESTED

1. Reagent grade sodium pentachlorophenate (Na PCP)

   A stock solution of 14,418 ppb sodium pentachlorophenate has been prepared according to the procedure of Alderdice (1963). The stock solution should be kept cool and in the dark prior to and after use. The recommended concentrations to be tested and the volumes of stock solution to be added for 30 liter bioassays are:

Concentration * (ppb)	ml of stock solution
1,000	2.08
500	1.04
100	0.21
50	0.105
10	0.021

2. Reagent grade dehydroabietic acid (DHAA)

   A stock solution of 6,667 ppm has been prepared by Dr. H. Rogers. The stock solution should be kept cool and in the dark prior to and after use. The recommended concentrations to be tested, and the volumes of stock solution to be added for 30 liter bioassays are:

* Note - These concentrations proved to be too wide a choice range. More intermediate concentrations should have been used close to the LC50, with choice left up to the investigator.

Concentration (ppm)	ml of stock solution
4 *	18.0
3	13.5
2	9.0
1	4.5
0.5	2.25

a. Replicate static bioassays are to be conducted at each of the five specified concentrations per toxicant tested.
b. Two control tanks, using only dilution water should be run in conjunction with each toxicant.
c. If possible, all bioassays for each toxicant should be run at the same time; i.e. this would mean a minimum of 5 conc. x 2 replicates + 2 controls = 12 bioassays/toxicant.

TEST FISH

1. Rainbow trout (*Salmo gairdneri* Richardson) will be principal test fish and will be used by all laboratories with the exception of I.P.S.F.C.[1]
2. Coho salmon (*Oncorhynchus kisutch* Walbaum) will be used for comparison purposed by all laboratories which have expressed an interest in using them.
3. Sockeye salmon (*Oncorhynchus nerka* Walbaum) will be the species tested by I.P.S.F.C.
4. Fish weight for all species should range between 1.0 - 3.0 grams per fish.

FISH HOLDING

1. All fish to be used for testing should be held in large aerated tanks at $10^{o} \pm 2^{o}C$.

* See note on previous page

[1] International Pacific Salmon Fisheries Commission

2. A minimum flow of water of 1/2 liter per minute per kilogram of fish is recommended for holding and acclimatization.
3. If acclimatization is required the temperature should be raised or lowered at a rate no greater than 1°C per day until 10°C is attained, and fish maintained at this temperature for at least 2 weeks prior to testing.
4. A reasonable sequence of darkness and light should be provided.
5. If fish mortality in the holding tank exceeds 1% per day, these fish sould not be used for testing; fish should be checked daily for disease symptoms and treated as necessary.
6. The fish should be fed once per day at a rate of 1-3% body weight/day preferably with 1/16" OMP[2] fish food. Feeding should be stopped 48 hours prior to, and for the duration of the test.

BIOASSAY TEST CONDITIONS

1. Glass or polyethylene containers with a minimum volume of 10 gallons, washed with alconox, sparkleen, comet[3],etc. and rinsed with distilled water should be used for all tests. The container should be covered with a transparent or translucent lid to prevent fish escapement (e.g. screen, plexiglass, etc.).
2. The total volume of test solution to be used for each tank should be 30 liters.
3. The fish loading density should be adjusted to 0.5 gm/l.
4. Preferably 10 fish should be used for each of the two replicates/concentration tested. If the fish weigh less than 1.5 gm each, two 30 liter bioassays/

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[2] O.M.P. - Oregon Moist Pellets.

[3] Laboratory detergents

conc. will suffice. If the fish are in the range of 1.5-3. gm each, more tanks will be required.

5. The water temperature should be maintained at $10^{o}C$ $\pm 2.0^{o}C$ for the duration of the tests and should be measured and recorded (in C^{o}) at the beginning and end of the test period, plus once daily.
6. The dissolved oxygen in each container should be maintained at greater than 90% saturation, using glass bubblers (glass rod or pipette) connected to an oil-free air source; D.O. levels should be measured and recorded at the beginning and end of the test period plus once daily.
7. The pH of the test solution should be adjusted to 7.0±.1 prior to commencement of the bioassays and should be measured and recorded daily. PH adjustments should be made with NaOH or HCl.
8. The water supply to be used for dilution should have a total hardness (expressed as $CaCO_3$) within the range of 5-20 ppm and the exact hardness reported.
9. A natural photoperiod or 12 hours of light and 12 hours of darkness should be used.
10. The maximum duration of the test will be 96 hours and all tests for a specific toxicant are to be run simultaneously.
11. As soon as all the fish have been placed in the test vessels, the tests are officially begun.
12. The test vessels are to be observed for mortalities at the following intervals: 30 minutes, 2,6, 24,48,72,96 hours; dead fish should be removed and the weights (gm), lengths (fork length), time of death and numbers of mortalities recorded.*
13. Death is to be regarded as the cessation of all movement by the fish, even after prodding.
14. Toxicity data should be reported as LT50's for individual concentrations and as an LC50 for each

* see text for comments on observation intervals

toxicant tested (Sprague, 1969).

15. All deviations from the recommended procedure should be reported in full when presenting the toxicity data.

APPENDIX II

ANALYSIS OF BIOASSAY DATA

Data analysis followed the recommendations of Sprague (1969, 1973) for determining incipient lethal concentrations (LC50's). The cumulative percentage mortality (probit scale) was plotted vs. time (log scale) for each toxicant concentration, as illustrated in Figure 1. From this plot the median survival time (LT50)required for one-half the test fish to die at each concentration was obtained. Application of the graphical procedures of Litchfield (1949) allowed calculation of 95% confidence limits for each median survival time. From these median survival times, a logarithmic toxicity curve was constructed (Fig. 2). This curve indicated that mortality data did become asymptotic to the time axis within 96 hours, indicating that a concentration threshold was present. Finally, the incipient lethal concentration (LC50) was determined from a log-probit plot of percentage dead at 96 hours (probit scale) vs. concentration(log scale) (Sprague, 1969). Following the nomographic procedure of Litchfield and Wilcoxon (1949) a line best fitting the points was drawn by minimizing the Chi^2 value of the line. From this approach the incipient LC50, complete with 95% confidence

limits and slope function, or S, for the line were obtained. If insufficient partial mortality data were present to allow use of the Litchfield and Wilcoxon procedure, LC50 was estimated from a log-probit plot of percentage mortality (probit scale) vs concentration (log scale). This latter approach did not allow accurate determination of the LC50 or calculation of its confidence limits, but provided an estimate only of incipient LC50.

Figure 1

Mortality of underyearling sockeye salmon in different concentrations of sodium pentachlorophenate. Data illustrate results of replicated bioassays conducted in fresh water with frequent observations of mortality. Two types of symbols are used for each concentration to avoid confusion between replicates and other concentrations. Data are all from the results of one laboratory.

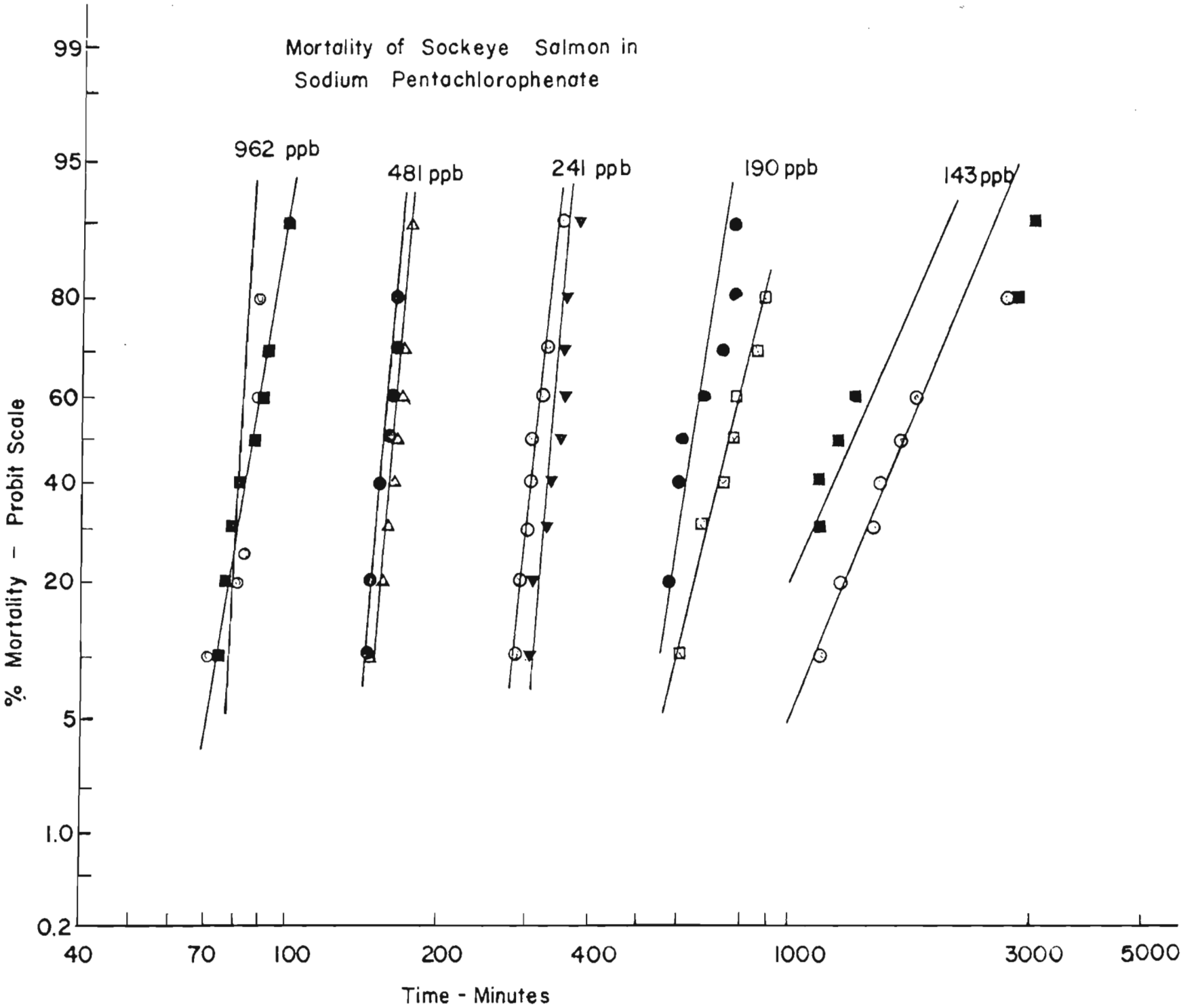
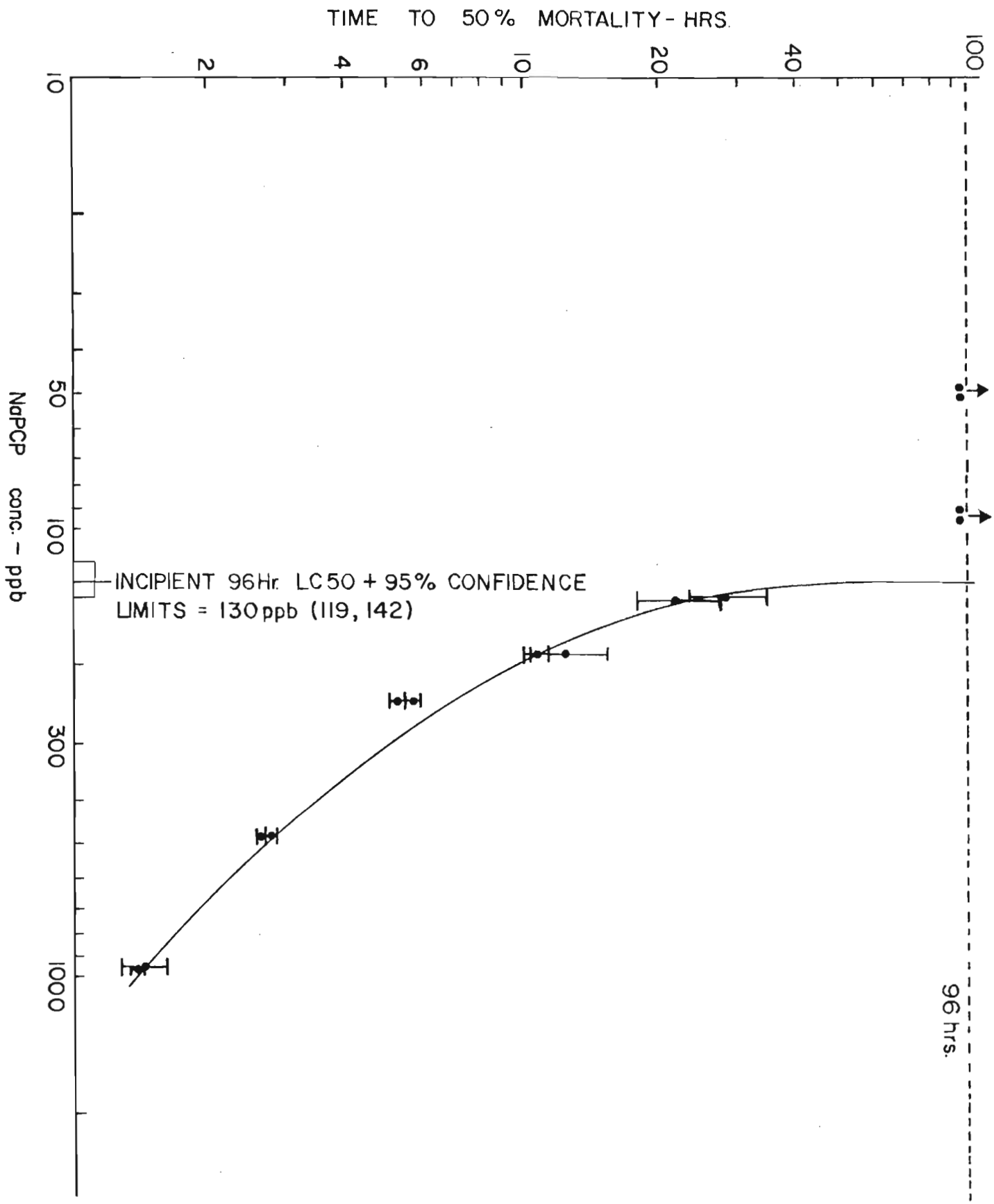

Mortality of Sockeye Salmon in
Sodium Pentachlorophenate
962 ppb
481 ppb
241 ppb
190 ppb
143 ppb
% Mortality - Probit Scale
99
95
80
60
40
20
5
1.0
0.2
40
70
100
200
400
1000
3000
5000
Time - Minutes

Figure 2

Plot of LT50's, as determined from Figure 1, complete with 95% confidence limits for LT50's to describe a toxicity curve. The incipient 96 hour LC50 as determined by nomographic analysis is indicated, complete with 95% confidence limits (lower and upper limits in brackets).


TIME TO 50% MORTALITY - HRS.
2
4
6
10
20
40
100
10
50
100
300
1000
NaPCP conc. - ppb
INCIPIENT 96Hr. LC50 + 95% CONFIDENCE LIMITS = 130 ppb (119, 142)
96 hrs.