Bull. Environm. Contam. Toxicol. 23,711-716 (1979)

Toxicity of Sodium Pentachlorophenate to Juvenile Chinook Salmon Under Conditions of High Loading Density and Continuous-Flow Exposure

G. K. Iwama[1] and G. L. Greer[2]

[1]*Ministry of Recreation and Conservation, Fish and Wildlife Branch, 400 — 1019 Wharf Street, Victoria, British Columbia, V8W 2Y9 and* [2]*Department of Fisheries and the Environment, Fisheries and Marine Service, Resource Services Branch, Pacific Environment Institute, 4160 Marine Drive, West Vancouver, British Columbia, V7V 1N6*

In describing methods for conducting toxicity tests with fish, SPRAGUE (1969, 1972) emphasized that the volume of test water per unit weight of fish is the variable of greatest importance affecting the results of static tests. His recommended guideline of 2-3 L of test water per g of fish per day is based on the oxygen requirements of fingerling trout. Since aeration of the test water is not advisable because of possible loss of toxicant through volatilization, this volume of test water would satisfy the respiratory requirements of most test fish except perhaps those of very small size. Further, it is suggested that the two other main factors affecting the test results, namely depletion of toxicant by fish uptake and deterioration of test water quality through accumulation of metabolic wastes, would be removed if the respiratory requirements of the test fish are met. Studies of bleached kraft mill effluent toxicity versus test water volume (DAVIS and MASON 1973) yielded results similar to SPRAGUE's (1969, 1972) recommendations; test volumes greater than 2.5 L per g of fish were required to eliminate the effect of volume on survival time in the effluent bioassays. Expressed in terms of loading density (g fish/unit volume test water), the recommended loading would be 0.3-0.5 g/L with a maximum of about 0.5 g/L desirable.

In studies of sublethal effects of toxicants where exposure to sublethal concentrations over an extended period of time is required, toxicant exposure by the continuous-flow method becomes the method of choice. Handling of the fish, as would be necessary for toxicant renewal in static exposure, is eliminated, toxicant and oxygen are continuously replaced and metabolic wastes removed. Even with continuous replacement of the test water, loading densities of about 0.5 g/L is recommended with the added feature that 90% replacement be achieved in 12 h or, if the toxicant is known to be unstable, 90% replacement in 8 h (SPRAGUE 1969, 1972). However, where a sublethal experiment requires a large sample size comprised of relatively large fish, the continuous-flow method of exposure may offer the added utility of permitting an increase in the loading density by increasing the replacement rate of the test water while at the same time achieving economy in tanks and space.

In the planning of a study (IWAMA 1977) to monitor, over a 40-day period, a number of hematological and blood biochemical responses of juvenile chinook salmon (Oncorhynchus tschawytscha) during progressive

0007-4861/79/0023-0711 $01.20

bacterial kidney disease and continuous-flow exposure to 0.5 and 0.55 96 LC50 of sodium pentachlorophenate (NaPCP), it was necessary to allocate one 185-L tank to each of the 6 treatment combinations. This was done to enable a high test water replacement time (90% in 1.5 h), a manageable schedule of replenishing the toxicant dispensing apparatus, and confinement of the experimental tanks to a small area to reduce the risk of kidney disease infection to a large fish holding facility nearby. With these constraints plus the size and numbers of fish needed to provide sufficient blood volume for the assorted analyses to be made at 4 d intervals, the experimental design yielded a loading density approximately 25-fold higher than the recommended 0.5 g/L. Contingent on carrying out this long term study was an estimate of the toxicity of NaPCP for juvenile chinook; however, the 96 h LC50 had not been reported in the literature. Although it seemed reasonable to assume the value might lie in the range 0.032-0.130 mg/L, as determined by the statis method for rainbow trout, Salmo gairdneri, coho, O. kisutch, and sockeye, O. nerka (DAVIS and HOOS 1975), it was uncertain an assumption of NaPCP toxicity in this range would hold true for chinook at such a high loading density, even with a high rate of test water replacement. It was, therefore, necessary to determine in a preliminary experiment the flow-through 96 h LC50 of NaPCP for juvenile chinook salmon at the loading density of 12-14 g/L that would be required in IWAMA's (1977) study. The premise on which an account of this bioassay is given here is as much to document the outcome of an extraordinary departure from the loading density recommended for toxicity testing as to report an estimate of NaPCP toxicity to juvenile chinook salmon.

MATERIALS AND METHODS

FISH: The chinook used in the bioassay of NaPCP were 10.3 $\pm$ 0.8 cm in length and weighed 11.25 $\pm$ 3.94 g (mean $\pm$ S.D.). They were reared in a 4000 L circular tank under natural photoperiod and in well water (10-11°C; pH 6.8-6.9; O_2 7-8.6 mg/L; hardness 54 mg/L $CaCO_3$). The diet consisted of Oregon Moist pellets fed daily. The stock was obtained from Qualicum Salmon Hatchery about 2 months before use.

APPARATUS: The bioassay tanks were 32.6 L glass aquaria set in a water bath at the diluent water temperature. The sides of the tanks were covered with black plastic and the tops covered with smoked Plexiglas to reduce external disturbances. The flows of diluent water (880 mL/min) and NaPCP stock solutions (3.9 mL/min) were collected in funnels set into the tanks covers and the mixture introduced near the bottom of the tank through tubular extensions on the funnels. The diluent water flow provided a 90% replacement time in 1.5 h (determined from Fig. 1 of SPRAGUE 1969). The NaPCP stock solutions were dispensed from 25-L, modified Mariotte bottles (LEDUC 1966).

A primary stock solution of 14.418 g/L NaPCP was prepared as described

by ALDERDICE (1963) but twice the quantity of NaOH was used because of difficulty with precipitation on dilution with water. From this primary stock, quantities required for the test concentrations in the bioassay tanks were added to the Mariotte bottles containing 1 L of water and 2 mL 5N NaOH, again because of difficulty with precipitation, while mixing continuously and then diluted to 25 L with continued mixing. The pH of these stock solutions was approximately 8.5. The test concentrations used in the bioassay were 0.06, 0.07, 0.08, 0.09, 0.13, 0.19 and 0.27 mg/L. The control tank received diluent water with equivalent amounts of NaOH added.

Each bioassay tank contained 40 fish. The average loading density was 13.8 g/L. The fish were acclimatized to the tanks for 2 weeks before exposure to NaPCP commenced. The fish received their usual food during this period except for the 2 days before starting the toxicant flows when feeding was stopped for the duration of the bioassay. Other conditions during the bioassay were: temperature, 11.8-12.0°C; pH, 7.0-7.1; oxygen, 83.0-84.6% saturation. The oxygen saturation levels exceeded the A-level protection recommended for freshwater salmonids (DAVIS 1975).

After the toxicant flows were started, the concentrations in the test tanks were allowed to equilibrate for 4 h, which was the time for approximately 99% replacement. At the end of this period, observations for mortality were made at 0.25, 0.5, 1.0, 2.0, 4.0 h and every 2.0 + 0.5 h thereafter. After the first mortality occurred, observations were made at more frequent intervals. Death was recorded as the time when opercular movements stopped.

The median survival time (LT50) was obtained from log-probit plots of cumulative percent mortality (SPRAGUE 1969). These data were used to construct a toxicity curve by plotting log-median survival times against log-toxicant concentration. The incipient lethal concentration (LC50) was determined from a log-probit plot of percentage dead at 96 h (probit-scale) against log concentration (SPRAGUE 1969). Following the nomographic procedure of LITCHFIELD and WILCOXON (1949) a line best fitting the points was drawn by minimizing the X^2 value of the line. This procedure yields a relatively accurate estimate of the incipient LC50 value and also allows the calculation of the 95% confidence limits.

RESULTS AND DISCUSSION

The incipient 96 h LC50 of NaPCP for juvenile chinook salmon at a loading density of 13.8 g/L was 0.078 mg/L; the 95% confidence limits were 0.057 and 0.110 mg/L (Fig. 1). The control fish appeared normal during the 2 weeks of acclimatization to the test tanks and throughout the bioassay period.

The NaPCP 96 h LC50 obtained in this continuous-flow bioassay lies in the mid-range of 96 h LC50 values determined for related species of juvenile salmonids by static method in an interlaboratory bioassay

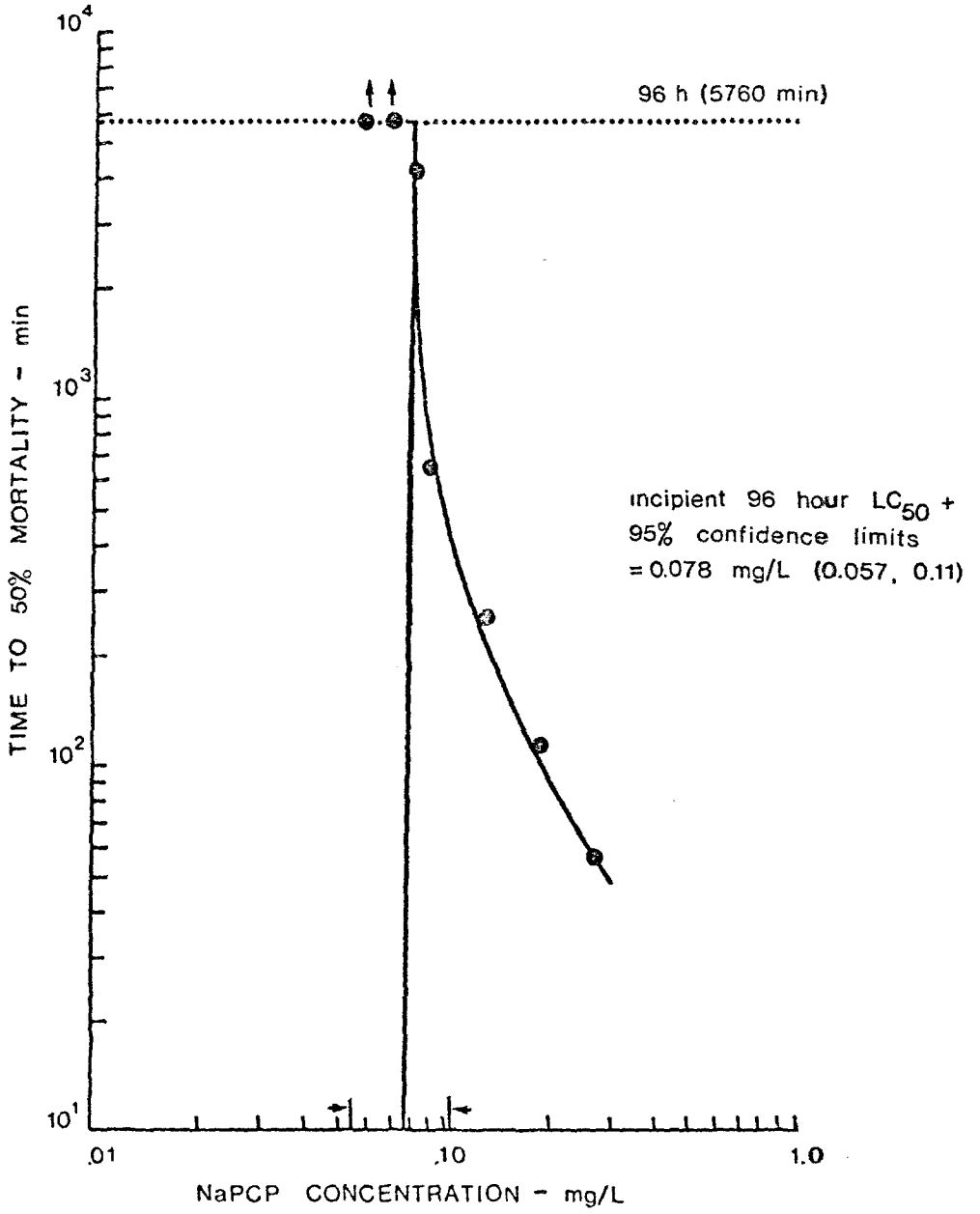

Fig. 1. Toxicity curve of sodium pentachlorophenate for juvenile chinook salmon exposed under continuous-flow conditions at a loading density of 13.8 g/L.

standardization study (DAVIS and HOOS 1975). A loading density of 0.5 g/L was used in that study and the ranges of NaPCP toxicity for the different species were: rainbow trout 0.047-0.106 mg/L; coho 0.032-0.092 mg/L; sockeye 0.05-0.130 mg/L. Possible sources of variability in the NaPCP toxicity values reported by the participating laboratories were not readily apparent.

Of the several variables that may modify the outcome of bioassay tests, eg. temperature, dissolved oxygen, pH, water hardness (SPRAGUE 1970), the pH of the bioassay solution is of particular importance in toxicity determinations of NaPCP because increasing amounts of the more toxic phenol moiety is formed as the pH drops below 7.5 (ALDERDICE 1963). In the present study the pH was 7.0-7.1, the same as the bioassay solution pH for seven of the nine inter-laboratory determinations by static bioassay. Similarly, temperature and water hardness were within the range of values reported in the standardization study. The main differences in the bioassay conditions in the present study were continuous solution replacement at a high rate (90% in 1.5 h) and the approximately 25-fold higher loading density. Given the range of NaPCP toxicity values reported for related juvenile salmonids (DAVIS and HOOS 1975), the 96 h LC50 of 0.078 mg/L for juvenile chinook at the high loading density of 13.8 g/L may be a reasonable estimate of NaPCP toxicity.

The possibility needs to be considered however, that the 96 h LC50 obtained in the present bioassay was a chance outcome. If the toxicant addition rate was too slow, reduction in the ambient concentration through fish uptake would result in a 96 h LC50 that is too high. The NaPCP concentrations in the test water were based on the stock toxicant concentration and the diluent water and toxicant flow rates. Possible reduction from theoretical in the ambient concentration because of fish uptake is not known. However, the percent saturation of oxygen in the water of the bioassay tanks with the system running but before the fish were placed in the tanks (85.4-90.5% sat.) and during the bioassay (83.0-84.6% sat.) indicates that only a small amount of oxygen was removed by the fish at the diluent water replacement rate used (90% in 1.5 h). Since the test water temperature was relatively constant (11.8-12.0°C), the levels of oxygen in terms of concentration would be proportional to the percent saturation values except for minor variations due to small changes in atmospheric pressure during the course of the experiment. SPRAGUE (1972) suggests that if the oxygen requirements of the fish are met during bioassays, the problems of toxicant depletion by the fish and waste accumulation in the test water will be resolved. It seems likely, therefore, that the toxicant addition rate calculated to give the desired ambient concentrations would not have been reduced by fish uptake.

SPRAGUE (1969) notes that one aspect of using a generous volume of test water in bioassays is simply provision of enough space for fish to swim in a reasonably free manner. At the loading density of 13.8 g/L, swimming space in the bioassay tanks clearly was limited. This crowding could have effected a stress making the fish more sensitive to the toxic action of NaPCP and have resulted in a 96 h LC50 that is erroneously low. Stress due to crowding has been

measured in juvenile coho at loading densities of 16-19.2 g/L in soft (2- mg/L as $CaCO_3$), 10°C water (WEDEMEYER, 1976). On the basis of loss of the feeding response which occurred at the lowest loading density, some degree of stress due to crowding was judged present. Since the loading density of the chinook in the present bioassay was similar to the loading density causing loss of feeding in the coho, it seems judicious to assume some level of stress was also present during the chinook bioassay and that this might have lowered the 96 h LC50 to some extent. Whether the resulting 96 h LC50 of 0.078 mg/L remains a good estimate of NaPCP toxicity to juvenile chinook cannot be fully evaluated until addition estimates made under more standard conditions become available for comparison.

REFERENCES

ALDERDICE, D. R.: Some effects of simultaneous variation in salinity, temperature and dissolved oxygen on the resistance of juvenile coho salmon (Oncorhynchus kisutch) to a toxic substance. Ph.D. Thesis. Univ. Toronto, Toronto, Ont. 177 p. (1963).

DAVIS, J. C.: J. Fish Res. Bd. Can. 32, 2245 (1975).

DAVIS, J. C. and R. A. W. HOOS: J. Fish. Res. Bd. Can. 32, 411 (1975).

DAVIS, J. C. and B. J. MASON: J. Fish Res. Bd. Can. 30, 1565 (1973).

IWAMA, G. K.: Some aspects of the interrelationship of bacterial kidney disease infection and sodium pentachlorophenate exposure in juvenile chinook salmon (Oncorhynchus tshawytscha). M.Sc. Thesis. Univ. B.C., Vancouver, B.C. 106 p. (1977).

LEDUC, G.: Le Naturaliste Canadien 93, 61 (1966).

LITCHFIELD, J. T. and F. WILCOXON: J. Pharmac. Exp. Ther. 196, 99 (1949

SPRAGUE, J. B.: Water Res. 3, 793 (1969).

SPRAGUE, J. B.: Water Res. 4, 3 (1970).

SPRAGUE, J. B.: The ABC's of pollutant bioassay using fish. A paper presented at the Symposium of Environmental Monitoring. June 27-28 1972 in Los Angeles, CA, at the Annual Meeting of the American Society for Testing and Materials. 43 p. (1972).

WEDEMEYER, G. A.: J. Fish. Res. Bd. Can. 33, 2699 (1976).