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Toxicity of Tri-*n*-Butyl-Tin to Chinook Salmon, *Oncorhynchus tshawytscha*, adapted to Seawater

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ABSTRACT

Short, J.W. and Thrower, F.P., 1987. Toxicity of tri-*n*-butyl-tin to chinook salmon, *Oncorhynchus tshawytscha*, adapted to seawater. *Aquaculture*, 61: 193–200.

The median lethal concentrations (LC_{50}s) of tri-*n*-butyl-tin oxide (TBTO) to juvenile chinook salmon, *Oncorhynchus tshawytscha*, adapted to seawater were determined in a static renewal bioassay. LC_{50}s were 54, 20, and 1.5 μg TBTO/l after exposures for 6, 12, and 96 h, respectively. LC_{50}s decreased logarithmically with time for exposures between 12 and 96 h. Also determined were the average tri-*n*-butyl-tin (TBT) concentrations in liver, brain, and muscle tissues of salmon that died during the bioassay: 7.0, 3.5, and 0.52 μg TBT/g wet weight tissue, respectively. TBT concentrations in liver, brain, and muscle tissues of salmon that survived until day 4 of the bioassay were 4300, 1300, and 200 times exposure concentrations, respectively. Our results implicate TBT exposure as the cause of death of chinook salmon exposed to TBT-treated marine net pens at one aquaculture facility.

INTRODUCTION

Tri-*n*-butyl-tin (TBT) compounds are widely used in the salmon aquaculture industry to retard fouling of net pens by marine organisms. Salmon at aquaculture facilities are raised to market size in marine net pens for 1–3 years, during which they gain most of their body mass. Nets must be cleaned periodically or chemically coated to retard fouling by marine organisms; fouling may reduce seawater exchange and result in fish kills. Antifoulants are much more economical than manual cleaning and are therefore preferred by the industry. Several antifoulant formulations are used to treat nets, but TBT compounds are among the most effective active ingredients. These compounds have low solubility in seawater (Maguire et al., 1983), are exceptionally toxic to marine fouling organisms (Hall and Pinkney, 1985), and can be formulated for slow release.

On several occasions, we observed high mortalities in groups of chinook

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salmon, *Oncorhynchus tshawytscha*, after transfer to newly TBT-treated marine net pens at an aquaculture research facility. The facility, operated by the National Marine Fisheries Service, is located at Little Port Walter (LPW), Alaska, near the southern end of Baranof Island. Affected fish were examined by the Alaska Department of Fish and Game Pathology Laboratory in Anchorage. Blood agar culture of kidney and spleen samples and microscopic examination of kidney and spleen smears were negative. No external or internal abnormalities were found. Exposure to TBT was therefore suspected as the cause of the mortalities.

To determine whether exposure to TBT could cause mortalities such as those observed at LPW, we determined the median lethal concentrations (LC_{50}s) of TBT to juvenile chinook salmon at several exposure periods, and the TBT concentrations in liver, brain, and muscle tissues of juvenile chinook salmon that died during the bioassay. These results were compared with those of Short and Thrower (1986) on muscle tissue concentrations of TBT in chinook salmon raised at LPW in TBT-treated marine net pens. Comparisons implicate TBT exposure as the cause of the mortalities observed at LPW.

METHODS

Bioassay animals

Chinook salmon used in the bioassay tests were raised for 1 year in fresh water and acclimated to seawater for 4 months before testing. Fish were transferred to tanks supplied with seawater (salinity, 28‰; temperature, 4°C; flow rate, 23 l/min), and were fed a diet of 3 mm Oregon Moist Pellets at a rate of 4% body weight daily until 5 days before the bioassay. Average wet weight of salmon used in the bioassay was 24.5 g (standard deviation = 16.43 g), and average fork length, 25.1 cm (standard deviation = 12.1 cm).

Bioassay

The bioassay was static, i.e., no water was replaced during the exposure period. Each of six 550-l fiberglass tanks contained one dose of TBT oxide (TBTO) and 10 randomly selected juvenile chinook salmon. A seventh 550-l fiberglass tank contained 10 similar chinook salmon, but no TBTO, and served as a control. The average ratio of wet weight of tissue to exposure volume was 0.0445 g/l. The seawater temperature was 4 ± 1°C throughout the exposure period. Solutions were aerated slowly to ensure adequate oxygen concentrations (above 80% saturation).

A solution of TBTO dissolved in 5.0 ml glacial acetic acid was mixed with seawater in the six exposure tanks, and 5.0 ml glacial acetic acid was mixed with seawater in the control tank. Salmon were then transferred by dip net to

the tanks. Dead and stressed salmon were noted at 6, 12, 24, 48, 72 and 96 h of exposure. Following 96 h of exposure, clean seawater was flushed through the exposure tanks at a rate of 23 l/min, and the survivors were observed for five additional days to determine any subsequent mortality. LC_{50}s were calculated using the method of Spearman and Kärber (Hamilton et al., 1977).

The solutions of TBTO in glacial acetic acid were prepared to give nominal TBTO concentrations of 2, 4, 8, 16, 32 and 64 μg TBTO/l exposure water. These doses were selected on the basis of trial exposures that determined approximate lethal doses. TBTO concentrations in exposure water were measured with atomic absorption spectrophotometry (AAS) immediately before salmon were placed in the solutions and, subsequently, once every 24 h. TBTO dose concentrations decreased to about 63% of those initially measured after 48 h of exposure; therefore, TBTO dissolved in 2 ml glacial acetic acid was added to each dose to increase the concentration to the original level. The 2-ml aliquot was added dropwise to the intake of a submersible pump in the exposure tank to minimize high localized concentrations of TBT. The TBTO dose concentration was measured just before and just after this addition of TBTO. We used the average of all AAS measurements for each nominal dose and exposure period to calculate the actual dose for that exposure period. Actual doses were used to calculate the LC_{50}s.

TBTO concentrations were measured by estimating the tin concentration of hexane extracts in the exposure water. One 50-ml aliquot of seawater was taken from each dose and extracted twice with two successive aliquots of 25 ml hexane each. Hexane extracts were combined and evaporated to dryness at 25°C on a rotary evaporator. The residue was taken up in 2–10 ml concentrated nitric acid and analyzed on a Perkin-Elmer model 5000 atomic absorption spectrophotometer equipped with a Zeeman background corrector. Concentrations of TBTO were estimated by comparison with standard concentrations of TBTO dissolved in hexane and processed similarly. With this method, recovery of TBTO from a TBTO concentration of 3 μg/l seawater was determined to be 95%.

Tissue sampling and analysis

Salmon that died during the bioassay were removed and stored frozen in glass jars. After thawing, all of the liver and brain and approximately 1 g of muscle tissue were dissected for analysis. Each tissue was mechanically homogenized and then extracted with hexane, and the tin concentration of the hexane extract was measured by AAS. Results are reported as if all the tin in the hexane extracts was tri-*n*-butyl-tin, although possibly some of the tin may have been di-*n*-butyl-tin. This method is more fully reported in Short and Thrower (1986).

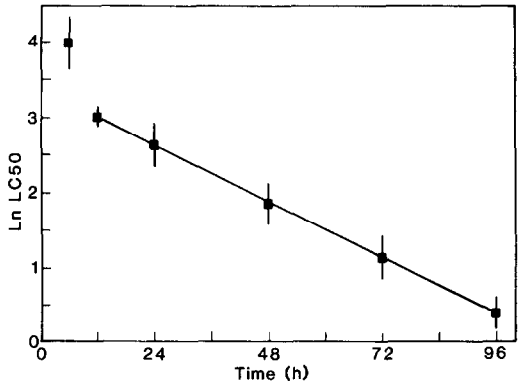

Fig. 1. Natural logarithm of TBT LC_{50} (μg TBTO/l) to juvenile chinook salmon, adapted to seawater, as a function of exposure time. Upper and lower ends of vertical bars indicate 95% confidence intervals. The solid line is derived from the linear regression of the natural logarithm of the LC_{50} with the exposure time.

RESULTS

All chinook salmon, except those exposed at the lowest dose, died during the 96-h bioassay, and none died in the clean water control tank during or immediately after the bioassay. At the lowest exposure dose, five salmon survived; of these, three died within the next 24 h in clean seawater. The logarithm of the LC_{50} decreased linearly with time between 12 and 96 h of exposure (Fig. 1). The natural logarithm of the LC_{50} fits the equation

$$\ln(LC_{50}) = -(0.031078)(T) + 3.363289; \qquad 12\text{ h} \leq T \leq 96\text{ h} \tag{1}$$

where T is the exposure time in hours. The measured 96-h LC_{50} was 1.5 μg TBTO/l seawater, whereas the measured 6-h LC_{50} was 54 μg TBTO/l seawater.

All salmon that died during the bioassay displayed the same series of progressive signs: darkened pigmentation, loss of stability, hemorrhage of the gills and fin insertions, defecation, and finally death. The lower the dose, the longer the exposure time was required for these symptoms to appear. Death usually occurred within 24 h of the onset of darkened pigmentation. The two survivors in the lowest exposure dose had darkened pigmentation at the end of the bioassay, but they returned to normal pigmentation within 24 h after being placed in clean seawater and apparently recovered from TBTO intoxication.

Concentrations of TBTO tended to decrease at all dose levels with time (Fig. 2). Dose levels declined to an average of 80% of the initially measured levels after the first 24 h of the bioassay and to an average of 63% after the first 48 h. Dose levels resumed their decline after TBTO was added to restore the desired concentrations.

Average concentrations of TBT in tissues of salmon that died during the

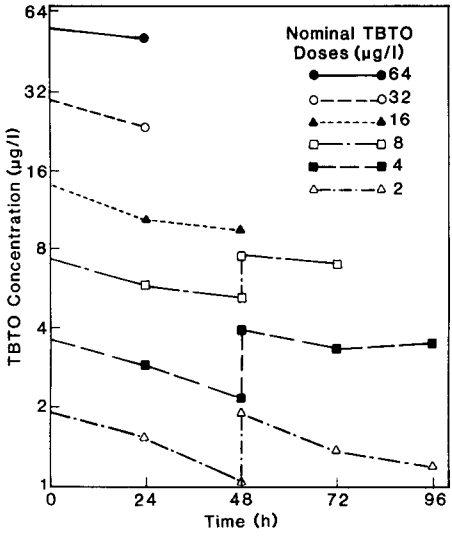

Fig. 2. TBT concentrations measured in bioassay doses as a function of time. TBT measurements were terminated in the higher doses after all the salmon in those doses died. The increase in measured TBT concentrations of the lower doses at 48 h of exposure is due to the addition of TBT to those doses at that time.

bioassay were highest in liver, intermediate in brain, and lowest in muscle tissues (Table 1). In liver and muscle tissues, the highest concentrations of TBT were in salmon killed by exposure to intermediate doses for intermediate exposure periods, and were about twice the concentrations found in salmon exposed to either high doses for brief periods or low doses for longer periods. In contrast, brain tissue concentrations of TBT were highest in salmon killed by exposure to high doses for brief periods.

We calculated apparent bioconcentration factors of liver, brain, and muscle tissues for salmon that died between 72 and 96 h of exposure to the lowest bioassay dose. These factors were 4300 for liver, 1300 for brain, and 200 for

TABLE 1

TBT concentrations in liver, brain, and muscle tissues of juvenile chinook salmon, adapted to seawater, that were killed by TBT exposure during the bioassay (n = number of individual salmon analyzed)

Tissue	Tissue concentration of TBT (µg/g) (±95% confidence interval)	n
Liver	7.44 ± 0.842	54
Brain	3.46 ± 0.330	53
Muscle	0.52 ± 0.213	49

muscle tissues, calculated as the ratio of the TBT concentration in tissue to the average exposure concentration of the lowest bioassay dose (1.49 μg TBT/l).

DISCUSSION

Juvenile chinook salmon are very sensitive to TBT poisoning in seawater. We found the 96-h LC_{50} of 1.5 μg TBT/l to be lower than any reported for fish in a recent survey of the literature on acute toxicity of organotins (Hall and Pinkney, 1985). The most significant difference between bioassay conditions in our experiment and those reported in Hall and Pinkney (1985) was that in ours, water temperature was lower (4°C), which may be the cause for some of the sensitivity observed.

TBT concentrations in salmon that died during the bioassay were nearly constant for all doses, suggesting that TBT continues to accumulate until a threshold concentration is reached in critical tissues and causes death. This conclusion is supported by our observation that salmon exposed to low doses of TBT displayed no intoxication symptoms until late in the bioassay. We speculate that the linear relationship between the logarithm of the LC_{50} and the exposure time (cf. Eqn. 1) indicates that significant mortalities may occur in salmon exposed for longer than 96 h to TBT concentrations lower than 1.5 μg/l.

The bioconcentration factors we measured are not equilibrium factors. Bioconcentration factors for salmon exposed to sublethal doses of TBT would probably be higher if the accumulation time was longer than in our study. However, our 96-h bioconcentration factors indicate that relatively brief exposure to TBT results in the accumulation of appreciable concentrations in salmon tissues.

Comparison of our results with those of Short and Thrower (1986) implicates TBT exposure as the cause of the mortalities observed at LPW. They found 0.82 ± 0.05 and 0.90 ± 0.10 μg TBT/g in muscle tissue of two groups of juvenile chinook salmon raised in TBT-treated marine net pens at LPW for 13 and 19 months, respectively. These TBT concentrations are slightly higher than those we found in muscle tissue of salmon that died during the bioassay (95% confidence interval, 0.9 ± 0.1 μg TBT/g vs. 0.52 ± 0.21 μg TBT/g). Salmon mortality at LPW was observed in the two groups of chinook salmon shortly after transfer to newly TBT-treated marine net pens: the mortality was over 50% of the population in one incident (Short and Thrower, 1986). The symptoms displayed by the stressed fish were similar to those we observed during the bioassay. The rate that TBT leaches from antifouling coatings is highest when the coatings are first exposed to seawater, and usually decreases exponentially with time (De la Court and De Vries, 1977). Salmon transferred to newly TBT-treated marine net pens would therefore be exposed to higher doses of TBT just after transfer. Short and Thrower (1986) also reported TBT con-

centrations ranging from 18 to 65 ng TBT/l in the surface waters of aged TBT-treated marine net pens. These concentrations were measured about a year after the mortality incidents, so it is plausible that the salmon were exposed to toxic doses of TBT when transferred to newly TBT-treated marine net pens. Exposure associated with transfer to newly TBT-treated marine net pens thus appears to be the most likely cause of salmon mortalities observed at LPW.

TBT leaching from treated marine net pens may cause adverse effects that are more subtle than intoxication symptoms or death. Growth in salmon could be affected by TBT; Chliamovitch and Kuhn (1977) have suggested that TBT inhibits metabolic pathways in rainbow trout, *Salmo gairdneri*. Therefore, we suspect that chinook salmon exposed for prolonged periods to sublethal doses of TBT in treated marine net pens may grow more slowly than those in untreated net pens due to the additional energy required to compensate for such stress. A similar effect has been demonstrated in salmon exposed to prolonged sublethal doses of the water-soluble fraction of crude oil (Moles and Rice, 1983). We know of no research indicating whether salmon raised in TBT-treated marine net pens may be more susceptible to disease. It is known, however, that low doses of TBT can impair the immune system of rats (Funahashi et al., 1980).

In summary, our results show that juvenile chinook salmon are very sensitive to TBT poisoning in seawater, that they rapidly accumulate TBT to high concentrations in tissues, and that lethal effects are dose and time dependent. For these reasons, TBT-treated net pens for salmon aquaculture applications should be used with caution.

ACKNOWLEDGEMENTS

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