TRI-N-BUTYL Tin CAUSED MORTALITY OF CHINOOK SALMON, ONCORHYNCHUS TSHWAYTSCHA, ON TRANSFER TO A TBT-TREATED MARINE NET PEN

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ABSTRACT

The median lethal concentrations (LC50's) of tri-n-butyl tin oxide (TBT) to juvenile chinook salmon, Oncorhynchus tshawytscha, adapted to seawater were determined in a static renewal bioassay. LC50's were 54, 20, and 1.5 μg TBT/l after exposure for 6, 12, and 96 h, respectively. LC50's decreased logarithmically with time for exposures between 12 and 96 h. Average tri-n-butyl tin (TBT) concentrations in liver, brain, and muscle tissues of salmon that died during the bioassay were 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 4,300, 1,300, and 200 times exposure concentrations, respectively. Average TBT concentrations in liver, brain, and muscle tissues of salmon surviving transfer to a TBT treated marine net pen that killed 8.5% of the salmon transferred were 9.56, 3.44 and 1.24 μg TBT/g wet weight tissue, respectively. Our results indicate TBT exposure was 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 to 3 years, during which they gain most of their body mass. Nets must be periodically cleaned or chemically coated to retard fouling by marine organisms; fouling will 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, are exceptionally toxic to marine fouling organisms, and can be formulated for slow release.

On several occasions, we observed high mortalities in groups of chinook 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 for disease agents, but none 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 (LC50'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 are compared with those of juvenile chinook salmon that had survived transfer to a TBT treated net pen at LPW that was suspected of killing some of the transferred fish due to TBT poisoning. Comparisons indicate 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°/o; temperature, 4°C; flow rate 23 l/min), and were fed a diet of 3 mm Oregon Moist Pellet 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 0.8445 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. LC50's were calculated using the method of Spearman and Karber.

The solutions of TBT in glacial acetic acid were prepared to give nominal TBT concentrations of 2, 4, 8, 16, 32, and 64 µg TBT/l exposure water. These doses were selected on the basis of trial exposures that determined approximate lethal doses. TBT 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. TBT dose concentrations decreased to about 63% of those initially measured after 48 h of exposure; therefore, TBT 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 TBT dose concentration was measured just before and just after this addition of TBT. We used the average of all measurements for each dose and exposure period to calculate the LC50 for each exposure period.

TBT 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 to 10 ml concentrated nitric acid and analyzed on a Perkin-Elmer model 5000 atomic absorption spectrophotometer equipped with a Zeeman background corrector. Concentrations of TBT were estimated by comparison with standard concentrations of TBT dissolved in hexane and processed similarly. With this method, recovery of TBT from a TBT concentration of 3 µg/l seawater was determined to be 95%.

**Results**

Chinook salmon died in all doses of TBT tested, but none died in the clean water control tank during or immediately after the bioassay. Only five salmon in the lowest exposure dose survived the bioassay; of these, three died within the next 24 h in clean seawater. The logarithm of the LC50 decreased linearly with time between 12 and 96 h of exposure (Fig. 1). The natural logarithm of the LC50 fits the following equation for a straight line for this exposure time period, using linear regression analysis:

\[
\ln(\text{LC50}) = -(0.031078)(T) + 3.363289 \quad (1)
\]

where T is the exposure time in hours. The measured 96-h LC50 was 1.5 µg TBT/l seawater, whereas the measured 6-h LC50 was 54 µg TBT/l seawater.

**Animals Surviving a Suspected TBT Poisoning Incident at LPW**

Chinook salmon were hatched in January 1985 and reared for 15 months before they were mistakenly transferred to a TBT coated net pen on 5 May 1986. Four hundred fish were transferred, having an average weight of 49 g. These animals had no known prior exposure to TBT. Within three days of transfer the fish displayed poor feeding response, darkened pigmentation, and tended to hang listlessly near the corners of the net pen. Thirty-four of the fish subsequently died. Personnel at LPW suspected TBT poisoning and verified that the net material actually was treated with TBT by tracing invoice records. On 20 May 1986 these fish were transferred to an untreated net pen, and no further mortalities occurred. Six of these fish were killed, frozen whole and sent to the Auke Bay Laboratory where they were stored frozen until analysis. Also sent were five cohorts of the TBT exposed fish that had never been exposed to TBT treated net pens as controls.

**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 were tri-n-butyltin, although possibly some of the tin may be di-n-butyltin. This method is more fully reported in Short and Thrower.

![Fig. 1. Natural logarithm of TBT LC50 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 LC50 with the exposure time.]
All salmon that died during the bioassay displayed the same series of progressive signs: darkened pigmentation, apathy, loss of stability, hemorrhage of the gills and fin insertions, defecation, and finally death. Salmon in the lowest doses did not display any symptoms until near the end of the bioassay period. 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 TBT intoxication.

Concentrations of TBT 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 TBT was added to restore the desired concentrations.

Average concentrations of TBT in tissues of salmon that died during the 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 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 4,300 for liver, 1,300 for brain, and 200 for 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).

Average concentrations of TBT in tissues of salmon that survived transfer into the TBT treated net pen at LPW are not significantly different from concentrations in corresponding tissues of salmon that died during the bioassay (Table 1). In contrast, much lower average TBT concentrations were found in tissues of salmon that were cohorts of the LPW salmon exposed to the TBT treated net pens (Table 1).

DISCUSSION

Juvenile chinook salmon are very sensitive to TBT poisoning in seawater. We found the 96-h LC₅₀ 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. The most significant difference between bioassay conditions in our experiment and those reported in Hall and Pinkney 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. The linear relationship between the logarithm of the LC₅₀ and the exposure time (cf. Equation 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 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.

The similarity of tissue TBT concentrations in salmon that died during the bioassay and salmon that survived transfer to the TBT treated net pen at LPW indicates that the transferred salmon were exposed to a nearly lethal dose of TBT. These results, together with the similarity of distress signs displayed by salmon tested in the bioassay and those transferred to the TBT treated net pen at LPW, indicate that TBT poisoning was the cause of death of the thirty-five salmon that died after
being transferred to the TBT treated net pen at LPW. The salmon that died represent the most sensitive individuals of the transferred group to TBT poisoning.

Table 1. Comparison of TBT concentrations in liver, brain, and muscle tissues of juvenile chinook salmon, adapted to seawater, that were killed by TBT exposure during the TBT bioassay, with survivors of a suspected TBT poisoning incident at LPW and with salmon from LPW that were not exposed to TBT. Concentrations are given as µg TBT/g muscle tissue (wet wt.), together with 95% confidence intervals. N = number of individual salmon analyzed.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>TBT of fish killed during bioassay</th>
<th>Transferred to TBT net pen at LPW</th>
<th>Control fish at LPW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>7.44 ± 0.84</td>
<td>9.56 ± 2.91</td>
<td>0.13 ± 0.24</td>
</tr>
<tr>
<td>Brain</td>
<td>3.46 ± 0.33</td>
<td>3.44 ± 2.54</td>
<td>0.12 ± 0.18</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.52 ± 0.21</td>
<td>1.24 ± 0.25</td>
<td>0.012 ± 0.007</td>
</tr>
</tbody>
</table>

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 have suggested that TBT inhibits metabolic pathways in rainbow trout, Salmo gairdneri. Chinook salmon exposed for prolonged periods to sublethal doses of TBT in treated marine net pens may therefore 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. Low doses of TBT can impair the immune system of rats, which suggests that salmon raised in TBT-treated marine net pens may be more susceptible to disease.

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.

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REFERENCES


