Bull. Environ. Contam. Toxicol. (1996) 56:788-795

Bulletin of Environmental Contamination and Toxicology

Effects of Tetrachloroguaiacol (*TeCG*) on the Osmoregulation of Adult Coho Salmon (*Oncorhynchus kisutch*)

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Received: 20 March 1995/Accepted: 16 November 1995

Fish are sensitive to many water-borne toxicants and alterations in fish physiology have been considered as potential diagnostic tools in environmental risk assessment. Fish gill epithelium is the dominant site of gas exchange, ionic regulation, acid-base balance, and nitrogenous waste excretion for fish (Hoar and Randall 1984) thereby serving a multitude of vital functions for these aquatic animals. In addition, gills are an important route for the uptake, biotransformation and excretion of toxicants (Evans 1987). In spite of their great physiological importance, gills are delicate structures, vulnerable to all kinds of environmental influences (Eddy 1981). Any damage to the gills will have immediate effects on ion homeostasis and will evoke compensatory osmoregulatory responses. Changes in plasma ion concentration, gill structure and Na^+/K^+ activated ATPase activity have been reported in fish exposed to sublethal levels of metals and organic chemicals (Heath 1990).

Tetrachloroguaicol (TeCG) is a xenobiotic released in water as a result of pulping processes. As the pulp and paper industry is a major industry in British Columbia, the impact of the paper effluent on the aquatic environment is of considerable interest. Fish undergoing the stresses caused by the chemicals in the effluents may not survive the disturbances and successfully make the physiological transition from hypoosmotic to hyperosmotic environment. The toxic action of tetrachloroguaiacol (TeCG), therefore, was investigated, determining its effect on osmoregulation of adult coho salmon (*Oncorhynchus kisutch*) in freshwater as well as during seawater challenge.

MATERIALS AND METHODS

Fish used in this experiment were adult coho salmon (*Oncorhynchus kisutch*) with a mean weight of 25.35 ± 0.65 g obtained from Capilano Hatchery at North Vancouver, British Columbia. Once the experiment started, the fish being tested were held in 70 L plastic garbage cans sitting in three large square tanks full of continuously running water to minimize temperature fluctuations. Control fish were also held in these cans to preclude the effect

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of plasticizers. There were three toxicant treatment groups and two controlgroups. For freshwater tests, one control and two TeCG treated groups were set up: (1) the freshwater group where fish were exposed to 100 µg/L TeCG (final concentration) for 3 d with the exposure solution partially changed (20 L/d) to ensure adequate toxicant loading. The dosed fish were then kept in clean freshwater during the 8 d depuration period; (2) Freshwater group where fish were exposed to 100 µg/L TeCG for 11-d with the exposure solution partially changed (20 L/d); (3) A control group was maintained in freshwater with no toxicant for 11 d. In the seawater challenge groups, the fish followed the same dosing process as in the freshwater group (3 d exposure to TeCG) but were transferred directly to ~30‰ seawater after toxicant exposure termination. Fish in the control group were simply held in toxicant free freshwater, still with 20 L water changed every day in the first three days of the experiment, and then were transferred to ~30‰ saltwater for the seawater challenge test, which lasted for 8 d. Fish in all groups were put in airstone equipped plastic garbage cans with either freshwater (except in the continuous exposure group) or seawater overflowing at the turnover rate of 2 hr during depuration or seawater challenge periods. There were 30 fish in each group and every garbage-can contained twelve fish with the density being approximately 8 g fish/L water. The experiment lasted for three weeks and the mean water temperature was 12.0 ± 1.2°C.

Before exposure to TeCG, six control fish were sampled and on each successive sampling day six fish were also sampled both in the control and the toxicant treated groups. The sampling days were as follows: exposure-day-3 (d3) in all groups; depuration-day-1,3,8 (dd1, dd3, dd8) in seawater challenge groups and all freshwater groups with the exception of the continuous exposure group where fish were sampled at exposure-day-4, 6 and 11 (d4, d6, d11). Fish sampling was achieved by terminal anaesthetization with MS222 (200 mg/L). Anaesthetization prior to sampling has been demonstrated to have no effect on plasma ion concentrations (Blackburn and Clarke 1987). Blood was sampled through the caudal ventral vein using heparinized 100 µL syringe and collected with 60 µL microhematocrit tubes. At the same time duplicate 20 µL blood samples were also taken into 10 mL small test tubes with Drabkin's solution for later hemoglobin measurement. The microhematocrit tubes were then centrifuged at 11,500 rpm for 3 min in a Damon IEC MB microhematocrit centrifuge and hematocrit (Hct) was measured in quadruplicate. Fish were then weighed and dissected. From each fish approximately 0.1 g gill and 0.3 g kidney tissues were taken and maintained in Eppendorff tubes with 500-µL SEI solution (0.3 *M* sucrose, 0.02 *M* EDTA, 0.1 *M* Imidazole) for the Na^+/K^+ activated ATPase assay (Zaugg 1982). The remaining fish carcasses along with the tissues and the plasma were stored at -80°C for later analysis.

Blood hemoglobin concentrations were determined by analyzing the hemolyzed blood and Drabkin's solution mixture at 540 nm using a Shimadzu

UV-160 visible recording spectrophotometer. Plasma sodium concentrations were measured on a Perkin-Elmer model 2380 atomic absorption spectrophotometer (aa). The plasma held in microhematocrit tubes under -80°C prior to ion level determination were thawed and diluted to within the linear range of the machine's detection. Gill and kidney Na^+/K^+ ATPase activity assays were conducted according to procedures described by Zaugg (1982).

All data are expressed as mean ± standard error. Statistical differences between treatment and control were determined using parametric one way ANOVA followed by a Dunnett's test for freshwater groups, and student t-test for seawater groups. Under conditions where equal variance test failed, the non-parametric equivalent was used as appropriate. A probability level of 0.05 was chosen as the limit of statistical significance.

RESULTS AND DISCUSSION

After a 3 d sublethal exposure to 100 µg/L TeCG, the experimental fish showed no change in either hematocrit (%) or hemoglobin concentration (g/dL) over the whole test duration (Figure 1 & 2). In the freshwater groups, plasma sodium concentration (mmol/L) was fairly stable until 4 days after the beginning of toxicant exposure, when a significant decrease ($p<0.05$) was seen (Figure 3). Then, plasma $[Na^+]$ climbed again from dd3 toward the end of the experiment during which time no statistical difference was recorded. In the seawater challenge groups, plasma $[Na^+]$ in fish exposed to TeCG was not significantly higher ($p<0.05$) than that in control group fish until 8 d after being transferred to toxicant free seawater (dd8) (Figure 3). The muscle moisture content (%) was significantly elevated ($P<0.05$) on ddl for partially dosed fish and d4 for continuously dosed fish (Figure 4) but later only those fish still exposed to TeCG had a significantly higher muscle moisture content compared with the control ones. The differences between treated and control animals in muscle moisture content were not maintained during the rest of the freshwater test. After the fish were transferred to seawater no significant difference was observed (Figure 4). Gill Na^+/K^+-ATPase activities (µmol Pi/mg Protein/hr) in these smolts were significantly increased ($p<0.05$) immediately after 3 d exposure to 100 µg/L TeCG. The enzyme activity, however, gradually returned to normal values in both the freshwater and the seawater groups, showing no difference from the control fish during the remaining 8 d of the experiment (Figure 5). The kidney Na^+/K^+-ATPase activity in the exposed fish, on the other hand, was not influenced by sublethal exposure to TeCG except on dd3 and dd8 in the seawater group and dd8 in freshwater group when the enzyme was significantly inhibited ($p<0.05$) (Figure 6).

Osmoregulatory disfunction was seen in TeCG treated coho salmon as indicated by the changes in plasma Na^+ concentrations (Figure 3) together

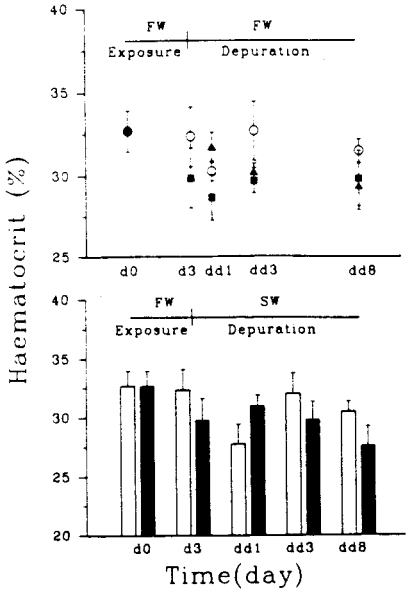

Figure 1. Haematocrit in coho salmon during TeCg exposure and depuration in freshwater (FW) and seawater (SW) transfer. Hollow circles and columns are controls, filled triangles and columns are 3-day-exposure groups and filled squares are continuous exposure groups.

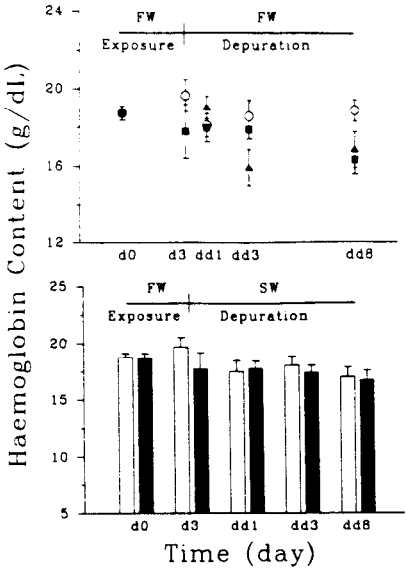

Figure 2. Haemoglobin concentration in coho salmon during TeCG exposure, depuration in freshwater (FW) and seawater (SW) transfer. Symbols as in Figure 1.

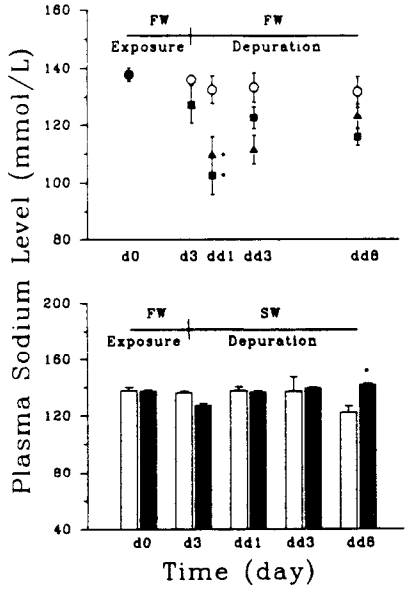

Figure 3. Plasma sodium level in coho salmon during TeCG exposure, depuration in freshwater (FW) and seawater (SW) transfer. The asterisk indicates a significant difference from control ($p<0.05$). Symbols as in Figure 1.

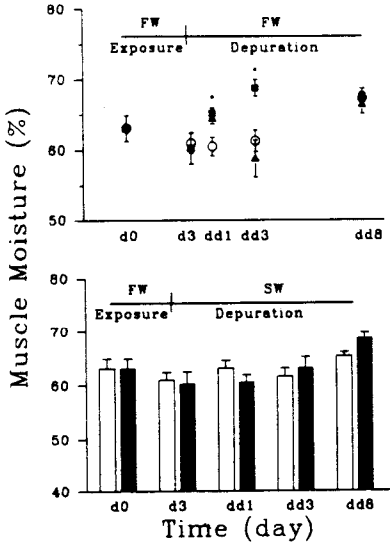

Figure 4. Muscle moisture content in coho salmon during TeCG exposure, depuration in freshwater (FW) and seawater (SW) transfer. The asterisk indcates a significant difference from control ($p<0.05$). Symbols as in Figure 1.

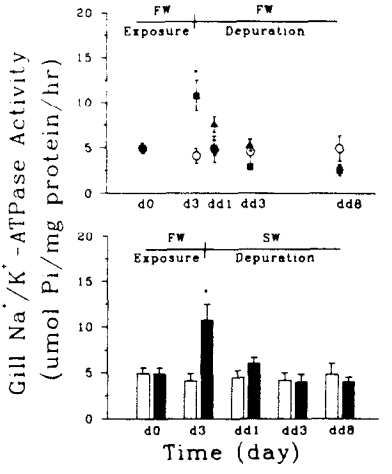

Figure 5. Gill Na^+/K^+ ATPase activity in coho salmon during exposure, depuration in freshwater (FW) and seawater (SW) transfer. The asterisk indicates a significant difference from control ($p<0.05$). Symbols as in Figure 1.

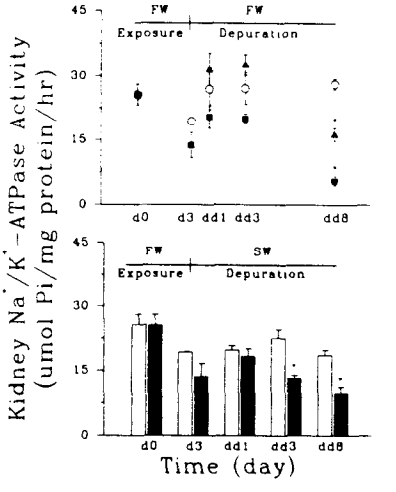

Figure 6. Kidney Na^+/K^+ ATPase activity in coho salmon during exposure. depuration in freshwater (FW) and seawater (SW) transfer. The asterisk indicates a significant difference from control ($p<0.05$). Symbols as in Figure 1.

with the corresponding changes in the muscle moisture contents (Figure 4). The plasma [Na^+] decrease in freshwater groups occurred at approximately the same time the muscle moisture content increased significantly in both groups. In freshwater, the reduction in plasma sodium (hemodilution) is associated with an increased water content in muscle. That is, the dilution is general and water entering the body is distributed to both blood and muscle tissues. The reasons responsible for either the plasma ion loss or gain shown respectively in the freshwater fish or the seawater challenged fish may be two fold, one important factor being a possible change in the permeability of the gill epithelium to water and ions, and the other factor being the impairment of active ion transport systems. In both freshwater and seawater fish, the diffusion gradient for sodium chloride is in the opposite direction to the osmotic diffusion of water across the gill epithelium. An increase in membrane permeability, which would increase water influx and ion efflux in freshwater fish and vice versa in seawater fish, could cause the decreased sodium level in plasma 4 days after the initial dosing in freshwater. group fish and the significantly higher plasma [Na^+] in TeCG treated fish 8 d after seawater transfer.

It appears that a wide range of organic xenobiotics are capable of affecting

fish osmoregulatory function by disturbing active ion transport (Evans 1987). The inhibition of gill Na^+/K^+ ATPase has been shown in different fish exposed to a variety of organic chemicals. Davis and Wedemeyer (1971) reported that the organochlorines, DDT, dicofol, and endosulfan inhibited rainbow trout gill Na^+/K^+ activated ATPase by 60 to 100% in vitro at concentrations between 10^{-5} and 10^{-4} *M.* It was found by Yang (unpublished data) that adult rainbowtrout (*Oncorhynchus mykiss*) exposed to 200 ug/L TeCG for 3 d showed an inhibition in the gill Na^+/K^+-ATPase activity. In this study a different species was used and the toxicant dose was lower (100 ug/L), which may be the reason why an upregulation in the gill Na^+/K^+-ATPase was observed (Figure 5). This increase in Na^+/K^+-ATPase activity was clearly due to the toxicant rather than a seawater challenge as the enzyme was affected in the same pattern in both freshwater and seawater. There have been reports of growth being stimulated in fish exposed to a toxic pollutant. Mason and Davis (1976) reported elevated growth of juvenile coho salmon when exposed to kraft pulpmill effluent. The stimulation was dose-dependent over the range tested. Although there was no direct evidence, a variety of hypotheses to explain this effect were proposed, including hormone analogs in the wood extracts that stimulate growth hormone and/or appetite. Another possibility is that hormesis occurred (Sansone and Martens 1981). The term "hormesis" refers to an overcompensation to some inhibitory challenge. The stimulation seen in the gill Na^+/K^+-ATPase activity could be described as a form of hormesis.

The kidney also plays an important role in osmoregulation, particularly in freshwater fish where glomerular filtration is high and extensive reabsorption takes place. According to the results of this study, kidney Na^+/K^+-ATPase was inhibited in the TeCG treated fish, although the response was delayed for 3 and 8 d, respectively, after the termination of toxicant dosing in the seawater and freshwater groups. Adult rainbow trout (*Oncorhynchus mykiss*) pre-exposed to the same chemical with a similar regime, but at a higher concentration (200 µg/L), showed no significant change in the kidney Na^+/K^+-ATPase activity even after a 8 d depuration period (Yang, unpublished data). The inhibition of the kidney Na^+/K^+-ATPase was similar in freshwater and seawater fish, indicating that the reduction was a direct effect of the toxicant on the ATPase rather than any secondary response to osmotic and ionic changes. Thus, it would seem that coho kidney Na^+/K^+-ATPase is more vulnerable than that of rainbow trout.

Plasma $[Na^+]$ levels of toxicant exposed fish were elevated above that of control only after 8 d in clean seawater. This increase in plasma $[Na^+]$ was associated with no change in gill Na^+/K^+-ATPase activity and a fall in kidney Na^+/K^+-ATPase activity. A decrease in kidney Na^+/K^+-ATPase would presumably impair Na^+ reabsorption, which would cause a fall in sodium levels in the body, rather than the observed increase. In freshwater, fish increases in gill Na^+/K^+-ATPase, which might be expected to cause an

elevation of plasma [Na^+], was followed in fact by the opposite, a drop in plasma sodium a day later. It would appear that the changes in plasma sodium are not brought about by changes in Na^+/K^+-ATPase in the body and, therefore, are due to increases in the passive flux of water and/or sodium. Thus, it would seem that the toxicant effects the permeability of the gills to sodium and water. We do not know if changes in sodium efflux or water influx were more important. There were no changes, however, in hematocrit, hemoglobin concentration or mean red cell volume, so there was little or no hemodilution, indicating that sodium loss was perhaps more important. The changes in Na^+/K^+-ATPase are also caused by the toxicant, as discussed earlier, the effect, however, may be to ameliorate the changes in plasma sodium caused by changes in sodium flux across the gills as the result of the action of the toxicant on the gill epithelium.

The manner and degree to which the sodium pump and membrane permeability are affected by these hydrophobic xenobiotics is not known. TeCG is a hydrophobic chemical, with a log K_{ow} (octanol/water partition coefficient) of 4.41. One hypothesis is that TeCG may change the bilayer structure of the gill epithelial phospholipid membrane due to their high lipid solubility, which may cause the change in epithelial osmotic and ionic permeability as well as the activity of Na^+/K^+-ATPase bound to the membrane of chloride cells. However, there is no clear evidence of the actual mechanism of action of these chemicals on the ion transport pump.

In conclusion, TeCG can adversely effect the osmoregulation of adult coho salmon smolts in freshwater and during seawater adaptation. The rationale behind this experiment was to see if fish subjected to organic chemicals in the pulp mill effluents will successfully make the transition from freshwater to seawater. It is noticeable that the osmoregulatory function in toxicant exposed coho salmon tended to recover in freshwater regardless of the dosing regime, *i.e.*, whether fish were only exposed for 3 days or continuously. The effect of TeCG exposure on fish seawater adaptability was observed only after several days in seawater. The impact of these toxicants, therefore, could be minimized if the exposed fish are left for several days to recover in the freshwater stream before they head for the ocean. In this regard, location of pulp mill plants to either freshwater or seawater sites, but away from the estuary might reduce their adverse impact on the local fishery as well as the regional aquatic ecosystems.

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