

**THE SAFETY OF CHLORAMINE-T TO VARIOUS LIFE STAGES OF
RAINBOW TROUT (*Oncorhynchus mykiss*)**

Study Protocol Number: BFTC-99-CHLT-TAS

Experiment Number 09: Rainbow trout juveniles tested at 14°C

Experiment start date: February 28, 2000

Experiment end date: March 24, 2000

Study Director

James D. Bowker, M.S.
U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
National Investigational New Animal Drug Office
4050 Bridger Canyon Road
Bozeman, MT 59715
Phone: 406-587-9265 ext. 126
FAX: 406-582-0242

Investigator

Daniel Carty, M.S.
U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
National Investigational New Animal Drug Office

ORIGINAL

Testing Facility

Bozeman Fish Technology Center
National Investigational New Animal Drug Office
4050 Bridger Canyon Road
Bozeman, MT 59715
Phone: 406-587-9265
FAX: 406-582-0242

Sponsor

David A. Erdahl, Ph.D
U.S. Fish and Wildlife Service
Bozeman Fish Technology Center
National Investigational New Animal Drug Office

James D. Bowker

J. D. Bowker 3/26/02

Daniel Carty

Daniel Carty 3/26/02

Executive Summary

The United States Fish and Wildlife Service's National Investigational New Animal Drug Office designed and conducted a target animal safety study (Study Protocol Number BFTC-99-CHLT-TAS) to generate data needed to obtain U.S. Food and Drug Administration (FDA) approval for the use of chloramine-T to control mortality in hatchery-reared salmonids diagnosed with bacterial gill disease or other Flavobacterial infections of the gills. The study consisted of 10 separate experiments in which groups of healthy fry, fingerling, or juvenile rainbow trout *Oncorhynchus mykiss* were acclimated to a water temperature of $\approx 8^{\circ}\text{C}$ or $\approx 14^{\circ}\text{C}$ and then exposed three times to chloramine-T at concentrations ranging from 0 to 100 mg/L. This report presents data generated during BFTC-99-CHLT-TAS-Experiment 09. The objective of Experiment 09 was to describe and evaluate histological effects of exposure to chloramine-T on three external (gill, skin, eye) and two internal (kidney and liver) fish tissues. These five tissues were identified by the FDA Center for Veterinary Medicine (CVM) and an independent histologist as the tissues most susceptible to damage from exposure to a water-borne chemical such as chloramine-T.

Experiment 09 lasted 26 d and consisted of a 7-d pre-exposure phase (experiment days 1 - 7), a 5-d exposure phase (experiment days 8 - 12), and a 14-d post-exposure phase (experiment days 13 - 26). During the experiment, groups of juvenile rainbow trout (the rainbow trout life-stage most sensitive to exposure to

chloramine-T; see results for BFTC-99-CHLT-TAS-Experiments 01 - 08 and 10) were exposed three times to chloramine-T at concentrations of 0, 20, 40, 60, 80, or 100 mg/L (i.e., 0x, 1x, 2x, 3x, 4x, or 5x the proposed maximum therapeutic treatment concentration of 20 mg/L). Each of the six exposure concentrations tested was administered in triplicate; consequently, 18 test tanks of fish ($n = 30$ fish per test tank) were used in the experiment. In the test tanks, groups of fish were acclimated to $\approx 14^{\circ}\text{C}$ water during experiment days 1 - 7 and then exposed to chloramine-T on experiment days 8, 10, and 12 (i.e., on alternate days). Chloramine-T exposures were administered as static-bath treatments that lasted 3 h each (i.e., three times longer than the proposed standard therapeutic treatment duration of 1 h). Completely randomized design procedures were used to allocate fish from holding tanks to test tanks. Randomized block design procedures were used to assign chloramine-T exposure concentrations to test tanks. Blinding techniques were employed to ensure that study participants involved in day-to-day data collection and the histologist did not know which test tanks received which exposure concentrations of chloramine-T.

Experiment 09 was conducted at a mean water temperature of 14.2°C ($\pm 1\text{SD} = 0.247$) and a mean dissolved oxygen concentration of 7.7 mg/L ($\pm 1\text{SD} = 0.582$). During each 3-h static-bath exposure period, both of these water quality parameters stayed within the acceptable limits specified in the study protocol ($14 \pm 2.5^{\circ}\text{C}$ and $8.5 \pm 2\text{ mg/L}$). Mean water hardness (209 mg/L) and alkalinity (168 mg/L), as well as all individual measurements of water hardness and alkalinity, were also within the

acceptable limits specified in the study protocol (210 ± 20 mg/L and 160 ± 20 mg/L, respectively). Mean pH (7.35) was within the acceptable range specified in the study protocol (7.7 ± 0.5), although one individual pH measurement (7.08) was slightly lower than the minimum acceptable pH (7.2) specified in the study protocol.

Colorimetric and high pressure liquid chromatography analyses revealed that the strength and purity of the chloramine-T used in the experiment was approximately 100%. Chloramine-T concentrations administered to each test tank on each exposure day were verified colorimetrically. For the 0-mg/L exposure group, measured chloramine-T concentrations that differed slightly from 0 mg/L were considered to be artifacts of the measurement process or the result of "color memory" in glassware or glassware caps from processing previous samples. For the 20-, 40-, 60-, 80-, and 100-mg/L exposure groups, all individual measurements of chloramine-T concentration were between -12.7% and +11.2% of their respective target concentrations. Moreover, for the 20-, 40-, 60-, 80-, and 100-mg/L exposure groups: (1) mean chloramine-T concentrations calculated for the overall experiment ($n = 5$ means) were between +0.2% and +2.5% of their respective target concentrations; (2) mean chloramine-T concentrations calculated for each exposure day ($n = 15$ means) ranged from -2.5% to +4.7% of their respective target concentrations; and (3) mean chloramine-T concentrations calculated for each test tank across all three exposure days ($n = 15$ means) were between -4.0% and +7.0% of their respective target concentrations. In all cases that could be statistically tested ($n = 33$ of 35), none of the mean chloramine-T

concentrations calculated for the overall experiment, for each experiment day, or for each test tank differed significantly (P -values > 0.05) from its respective target concentration. Quality control monitoring (also done colorimetrically) indicated that chloramine-T dose-verification procedures had been carried out with reasonable precision and accuracy during the course of the experiment.

To describe and evaluate effects of exposure to 0, 20, 40, 60, 80, or 100 mg/L chloramine-T on gill, skin, eye, kidney, and liver tissues of juvenile rainbow trout, fish were collected and sampled during six periods. Fish from the reference population (i.e., pre-exposure group) were collected and sampled on experiment days 4 and 5. "Moribund-appearing" test fish (i.e., fish that appeared to be near death) were collected and sampled on experiment days 8 and 10 (during or at the end of the first and second chloramine-T exposure periods). "Healthy-appearing" test fish (i.e., fish that appeared to behave normally) were collected and sampled on experiment days 12 (during or at the end of the third exposure period), 19 (7 d after the third exposure period), and 26 (14 d after the third exposure period). For each of the six exposure groups tested in this experiment, total mortality was calculated by adding the number of "moribund-appearing" test fish collected for fish health and histology sampling to the number of dead fish found in and removed from the test tanks.

Fish sampled from the reference population (i.e., pre-exposure fish) were found to be generally healthy and to have no serious histological problems; thus, it was

inferred that the fish used in the experiment had no pre-existing conditions that could have adversely affected the experiment's outcome. All test fish in the 0-, 20-, and 40-mg/L exposure groups remained "healthy-appearing" throughout the experiment, and percent total mortality for each of these groups was calculated to be 0.0%. Test fish collected from the 0-mg/L exposure group on experiment days 12, 19, and 26 were found to be generally healthy and to have no serious histological problems. Test fish collected from the 20- and 40-mg/L exposure groups on experiment days 12, 19, and 26 were found to be generally healthy; however, one sub-lethal, moderate pathology was evident in the gill tissue of one 20-mg/L test fish, and a small number of sub-lethal, moderate pathologies were evident in the gill and kidney tissues of a few 40-mg/L test fish. In these two exposure groups, as well as in the 60-, 80-, and 100-mg/L exposure groups, moderate and severe pathologies observed in gill tissue were considered to be immediate changes caused by exposure to chloramine-T, whereas moderate and severe pathologies observed in kidney tissue were considered to be delayed responses by the kidney to "clean-up" some of the damage done to the gills.

A mixture of "moribund-appearing" test fish and "healthy-appearing" test fish were collected from the 60-, 80-, and 100-mg/L exposure groups, and the percent total mortality for each of these groups was calculated to be 4.4, 60.0, and 98.9%, respectively. "Moribund-appearing" test fish collected on experiment days 8 and 10 displayed a variety of moderate and severe pathologies in their gill and kidney tissues, and one of these fish also displayed a moderate pathology in its liver tissue. This one

moderate liver pathology was considered to be a delayed change because it was only observed after the second chloramine-T exposure had been administered. "Healthy-appearing" test fish collected from the 60-, 80-, and 100-mg/L exposure groups on experiment days 12, 19, and 26 displayed fewer pathologies than were evident in "moribund-appearing" test fish; thus, it appeared that test fish that survived the first two chloramine-T exposures were little affected by the third chloramine-T exposure and were recovering from the toxic effects of chloramine-T. In this experiment, as well as in the nine other experiments conducted under Study Protocol Number BFTC-99-CHLT-TAS (Experiments 01 - 08 and 10), nearly all of the mortality that was observed occurred during or within 20 - 24 h of the first chloramine-T exposure period.

Based on the results of the fish health and histology evaluations, combined with observational and percent total mortality data, it was concluded that:

1. The proposed maximum therapeutic treatment concentration of 20 mg/L chloramine-T, when administered as a static-bath treatment three times on alternate days, is safe for use on juvenile rainbow trout reared at a water temperature of $\approx 14^{\circ}\text{C}$;
2. For juvenile rainbow trout reared at a water temperature of $\approx 14^{\circ}\text{C}$, the margin of safety for exposure to chloramine-T extends to at least 40 mg/L, but is, for practical purposes, less than 60 mg/L;

3. Juvenile rainbow trout are probably most susceptible to the toxic effects of relatively high concentrations of chloramine-T (i.e., ≥ 60 mg/L) the first time they are exposed to it; and

4. Juvenile rainbow trout that survive exposure (especially the first exposure) to relatively high concentrations of chloramine-T (i.e., ≥ 60 mg/L) are capable of recovering from the toxic effects of such exposure.