Date of Approval: JUL 2 8 2008

SUMMARY OF SAFETY AND EFFECTIVENESS DATA IN PMF 005893

Chloramine-T

Soluble powder for immersion
Freshwater-reared salmonids

For the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp.

Sponsored by:

United States Department of the Interior
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program

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I. GENERAL INFORMATION:

A. File Number: PMF 005893

B. Sponsor: United States Department of the Interior
United States Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
4050 Bridger Canyon Road
Bozeman, MT 59715

C. Proprietary Name: To be determined

D. Established Name: Chloramine-T

E. Pharmacological Category: Antimicrobial (disinfectant)

F. Dosage Form: Reconstituted powder for immersion

G. Amount of Active Ingredient: Benzene sulfonamide, N-chlora-4-methyl, sodium salt (99.0 to 100.0% pure)

H. How Supplied: Chloramine-T is a white crystalline powder that is packaged in a variety of quantities (i.e., 5 kg and 25 kg) and also different packages (i.e., plastic tub, plastic buckets, drums)

I. How Dispensed: Over-the-counter (OTC)

J. Dosage: 12 to 20 mg chloramine-T per liter of water in continuous flow water supply or as a static bath for 60 minutes on alternate or consecutive days for three treatments.

K. Route of Administration: Immersion bath

L. Species/Class: Freshwater-reared salmonids

M. Indication: For the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp.

II. EFFECTIVENESS:

The data summarized in this section are publicly available and contained in Public Master Files 005893 and 005637 and Investigational New Animal Drug Files 004000 and 009321 which were compiled by the United States Department of the Interior, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership Program and the U.S. Geological Survey, Upper Midwest Environmental Sciences Center.

A. Dosage Characterization:

The primary effect of chloramine-T results from localized action at the topical site of administration. The concentration of the active drug at the topical site is a function of the administered concentration, exposure period, and water conditions. These three conditions and the sensitivity of the pathogen to the drug are considered the primary determinants of effectiveness.

Initial doses for the control of mortality due to bacterial gill disease in freshwater-reared salmonids were selected based on published literature and the experience of managers of state and federal fish hatcheries. An effective dose range of 12 to 20 mg/L administered as a static bath every other day for three treatments was determined to be effective in pilot testing. Dose confirmation studies at the low end of the dose range, 12 mg/L, were conducted as described below.

In addition to treatment in a static bath, administration in a flow-through system was also characterized to be equally as effective when the same treatment regimen was followed. To determine the ability to accurately dose and maintain concentrations of the drug in a flow-through system a special study was conducted as described below.

B. Substantial Evidence:

Substantial evidence of effectiveness was demonstrated in three clinical field studies for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp. Additionally, a study to measure concentrations of the drug in a flow-through system was conducted in a common concrete raceway.

1. Clinical Effectiveness Field Study – Study 4000-1-04

Title: "Efficacy of Chloramine-T to Control Mortality Caused by Bacterial Gill Disease (BGD) in Fingerling Rainbow Trout"

Study Director: James D. Bowker

Study Location: Hotchkiss National Fish Hatchery
Hotchkiss, Colorado

General Design of the Study Design:

a. Purpose: To evaluate the effectiveness of chloramine-T as a bath at a concentration of 12 mg/L for 60 minutes every other day for 3 treatments for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in rainbow trout.

b. Animals: Approximately 6000 rainbow trout fingerlings

c. Test article/Controls: Chloramine-T. The control fish were unmedicated.

d. Study Design: The fish were randomly transferred into each of 6 test tanks (1000 fish/tank). Untreated control and chloramine-T treatment groups were tested in triplicate. Before treatment, 5 fish from each tank were evaluated for the presence of BGD. Sixty-minute chloramine-T treatments of 12 mg/L were administered as a static bath on Days 1, 3, and 5. The study duration was 19 days. Chloramine-T concentrations in the treated tanks were confirmed during treatments. Mortalities were counted twice daily. Fish were examined (body surface, fins, gills and internal organs) and kidney inocula were cultured for the presence of systemic bacteria. Stained gill imprint slides were examined. Stained gill squash slides were examined from 5 fish prior to the first treatment, on Day 5 following the last treatment, and on Day 19.

e. Variables Measured: Mortality and water quality parameters

Statistical Analysis: Mortality rates were analyzed using generalized linear models (GENMOD procedure), using a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included.

Results: Mortality results are included in the following table.

Table 1. Percent cumulative mortality from Day 1 through 19.

Chloramine-T Concentration	Cumulative Mortality	Percent Cumulative Mortality	LSMean (GENMOD)
0 mg/L	773/3000	25.8	-1.0581
12 mg/L	171/3000	5.7	-2.8060
		p-value	<.0001

Temperature and dissolved oxygen (DO) were consistent throughout the study for all test tanks and mean values were 13.8 °C and 6.6 mg/L, respectively. Mean water hardness and pH values, based on measurements made on Days 1, 2, and 4, were approximately 396 mg/L (as $CaCO_3$) and 7.8, respectively.

Conclusion: Chloramine-T administered at a concentration of 12 mg/L for 60 minutes every other day for 3 treatments is effective in the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in rainbow trout.

2. Clinical Effectiveness Field Trial – Study 4000-1-003

Title: "Efficacy of Chloramine-T to Control Mortality in Apache Trout (*Oncorhynchus apache*) Caused by Bacterial Gill Disease Associated with Flavobacters"

Study Director: James D. Bowker

Study Location: Alchesay-Williams Creek National Fish Hatchery
Whiteriver, Arizona

General Design of the Study:

a. Purpose: To evaluate the effectiveness of chloramine-T as a bath at a concentration of 12 mg/L administered for 60 minutes every other day for 3 treatments for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in apache trout.

b. Animals: Approximately 12,970 apache trout fingerlings

c. Test article/Controls: The control fish were unmedicated.

d. Study Design: The fish were randomly transferred into each of 6 test tanks (approximately 2340 fish/tank). Untreated control and chloramine-T treatment groups were tested in triplicate. Sixty-minute chloramine-T treatments of 12 mg/L were administered as a static bath on Days 1, 3, and 5. The study duration was 19 days. Chloramine-T concentrations were confirmed during treatments. Mortalities were counted twice daily. Before treatment and at the end of the study, 5 to 6 fish from each tank were evaluated for the presence of BGD. Fish were examined (body surface, fins, gills, and internal organs) and kidney inocula were cultured. Stained gill squash slides were examined from 5 fish before treatment and after the third treatment.

e. Variables Measured: Mortality and water quality parameters

Statistical Analysis: Mortality rates were analyzed using generalized linear models (GENMOD procedure), using a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included.

Results: Mortality results are included in the following table.

Table 2. Percent cumulative mortality from Day 1 through 19.

Chloramine-T Concentration	Cumulative Mortality	Percent Cumulative Mortality	LSMean (GENMOD)
0 mg/L	6397/6524	98.1	3.9194
12 mg/L	2508/6446	38.9	-0.4512
		p-value	<.0001

The overall mean measured concentration of chloramine-T among treated tanks was 11.6 mg/L. Temperature and dissolved oxygen (DO) were consistent throughout the study for all test tanks and mean values were 11.6 °C and 7.4 mg/L, respectively. Mean water hardness and pH values were approximately 45 mg/L (as $CaCO_3$) and 7.1, respectively.

Conclusion: Chloramine-T administered at a concentration of 12 mg/L for 60 minutes every other day for 3 treatments is effective in the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in apache trout.

3. Clinical Effectiveness Field Study – Study 4000-1-002

Title: "Efficacy of Chloramine-T to Control Mortality Caused by Bacterial Gill Disease Associated with Flavobacters in Fall Chum Salmon (*Oncorhynchus keta*)"

Study Director: James D. Bowker

Study Location: Quilcene National Fish Hatchery
Quilcene, Washington

General Design of the Study:

a. Purpose: To evaluate the effectiveness of chloramine-T as a bath at a concentration of 12 mg/L for 60 minutes every other day for 3 treatments for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in fall chum salmon.

b. Animals: Approximately 96,594 fall chum salmon

c. Test article/Controls: Control fish were unmedicated.

d. Study Design: The fish were randomly transferred into each of 6 test tanks (approximately 13,419 to 19,248 fish/tank). Untreated control and chloramine-T treatment groups were tested in triplicate. Before treatment,

5 fish from each tank were evaluated for the presence of BGD. Sixty minute chloramine-T treatments of 12 mg/L were administered as a static bath on Days 1, 3, and 5. The study duration was 19 days. Chloramine-T concentrations were confirmed during treatments. Mortalities were counted twice daily. Fish were examined (body surface, fins, gills, and internal organs) and kidney inocula were cultured. Stained gill squash slides were examined from 5 fish before treatment and on Days 6, 11, and 22.

e. Variables Measured: Mortality and water quality parameters

Statistical Analysis: Mortality rates were analyzed using generalized linear models (GENMOD procedure), using a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included.

Results: Mortality results are included in the following table.

Table 3. Percent cumulative mortality from Day 1 through 19.

Chloramine-T Concentration	Cumulative Mortality	Percent Cumulative Mortality	LSMean (GENMOD)
0 mg/L	48,067/48,241	99.6	5.6213
12 mg/L	3907/48218	8.1	-2.4285
		p-value	<.0001

The overall mean measured concentration of chloramine-T among treated tanks was 12.7 mg/L. Temperature and dissolved oxygen (DO) were consistent throughout the study for all test tanks and mean values were 10.0 °C and 9.9 mg/L, respectively. Mean water hardness and pH values were approximately 38 mg/L (as $CaCO_3$) and 7.3, respectively.

Conclusion: Chloramine-T administered at a concentration of 12 mg/L for 60 minutes every other day for 3 treatments was effective in the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in fall chum salmon.

4. Administration Method Justification

The above studies were done with chloramine-T administered as a static bath. Under standard hatchery conditions, administering treatment in a static bath is not always preferred because of potential degradation of water quality conditions during treatment. The following study was designed to demonstrate that a target dose of chloramine-T can be achieved and maintained during flow-through treatment.

Title: "Analytical Verification of Chloramine-T to Confirm Target Dosage in a Bath Solution Administered Using a Flow-Through Treatment Method"

Study Director: James D. Bowker

Study Location: US Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

General Design of the Study:

a. Purpose: To determine if concentrations of chloramine-T measured in water samples taken from various locations within a raceway at various times during a one-hour treatment are within 25% of the target dose of 12 mg/L.

b. Animals: None

c. Test article/Controls: Chloramine-T. There were no control animals used.

d. Study Design: Two 58 ft x 6 ft x 3.7 ft outdoor raceways were used. The water-volume of each raceway was approximately 580 ft^3. Each raceway was tested twice for a total of four trials. One raceway was tested daily for two days and the other was tested daily on the following two days. With the water flow off, chloramine-T was added and mixed manually to establish an initial concentration of 12 mg/L. The amount of chloramine-T used was calculated using the following equation from Piper et al. (1982).

   Chloramine-T (g) = target dose (mg/L) x water volume (gal) x 0.00378 (L-gal conversion)

   Water flow was resumed at a predetermined flow rate. A chloramine-T solution was metered into the raceway inflow water. The amount of chloramine-T used was calculated using the following equation from Piper et al. (1982).

   Chloramine-T (g) = target dose (mg/L) X water flow rate (gal/min) X treatment duration (min) X 0.00378 (L-gal conversion)

   Water samples were collected at 0, 30, and 60 minutes during the treatment period using a central aligned square grid systematic sampling scheme. Samples were collected from the raceway at the head-end, middle, and tail-end; the surface, middle and bottom; and along each side and the midline.

e. Variables Measured: Chloramine-T concentration

Statistical Analysis: Two one-sided t-tests were performed to determine whether the mean concentration was a) above 9 mg/L and b) below 15 mg/L. These tests were done for the mean taken over all trials and time points (0, 30, and 60 minutes) after treatment, for the mean of each trial, and for the mean at each time point after treatment.

Results: The overall mean chloramine-T concentration from the four trials was 10.8 mg/L. The mean concentrations of chloramine-T for all studies and in all strata sampled were within 75 to 125% of the target concentration of 12 mg/L. The mean chloramine-T concentrations at 0, 30, and 60 minutes were 11.7, 10.5, and 10.2 mg/L, respectively. The mean chloramine-T concentration over all trials and time points, in each trial, and at each time point were statistically significantly higher than 9 mg/L and lower than 15 mg/L ($p < 0.0001$ for all tests).

The highest mean chloramine-T concentration was at the head-end of the raceway at 0 minutes, and the lowest mean chloramine-T concentration was at the head-end of the raceway at 60 minutes. Mean chloramine-T concentrations measured at 0 minutes were consistently higher than mean chloramine-T concentrations measured at 30 or 60 minutes. The mean chloramine-T concentrations from all four trials at the surface, middle, and bottom of the raceway were 10.7, 11.0, and 10.7 mg/L, respectively. The mean chloramine-T concentrations from all four trials at the right-hand side, midline, and left-hand side of the raceway were 10.7, 10.9, and 10.8 mg/L, respectively.

Conclusion: The target dose of chloramine-T can be accurately administered for a 60-minute treatment duration by initially adding chloramine-T to static water to obtain the target concentration followed by metered administration in a flow-through system.

III. TARGET ANIMAL SAFETY:

A. Target Animal Safety Studies in Rainbow Trout

Title: "The Safety of Chloramine-T to Various Life Stages of Rainbow Trout (*Oncorhynchus mykiss*)"

Study Director: James D. Bowker, MS

Study Location: US Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

General Design of the Study:

a. Purpose: To demonstrate the safety of chloramine-T administered as a bath to fry, fingerling, and juvenile life-stages of rainbow trout, *Oncorhynchus mykiss*.

b. Animals: Fry, fingerling, and juvenile rainbow trout

c. Test article/Controls: Chloramine-T. Control fish were unmedicated.

d. Study Design: The life stage, number of fish, chloramine-T concentrations and water temperature during studies are included in the following table.

Table 4. Life stage, number of fish, chloramine-T concentrations, and water temperature during a series of safety studies.

Study Number	Life Stage	Chloramine-T Concentration (mg/L)	Number of Fish (*)	Water Temp. (°C)
1	Fry	0, 20, 60, 100	1200 (100)	8
2	Fry	0, 20, 60, 100	1200 (100)	14
3	Juvenile	0, 20, 60, 100	480 (40)	8
4	Juvenile	0, 20, 60, 100	360 (30)	14
5	Fingerling	0, 20, 40, 60	600 (50)	8
6	Fingerling	0, 20, 30, 40, 50, 60	900 (50)	14
8	Juvenile	0, 50, 60, 70, 80, 100	540 (30)	14
10	Juvenile	0, 20, 40, 60, 80, 100	540 (30)	14

*fish per tank

The same protocol was used for the eight studies. Chloramine-T was administered in static baths in multiples of a 20 mg/L concentration. Chloramine-T was administered once daily every other day for 3 treatments, 3 hours per treatment, with one exception. During Study Number 10, treatments were administered once daily on 3 consecutive days. Mortality observations were made every 30 minutes during treatments. Approximately 1 to 2 hours into each of the treatments, water samples were collected for analysis of chloramine-T concentration.

e. Variables Parameters Measured: Mortality, chloramine-T concentrations, and water quality parameters

Statistical Analysis: The cumulative mortality rates for both time periods in each study (24 hours and 14 days post-treatment) were analyzed using generalized linear models (GENMOD procedure in SAS). The model included the fixed effect treatment. A binomial error distribution and logit link function were used. The experimental unit was the tank. Treatment comparisons were made between the active treatment groups and the control group for those treatment groups that had at least one mortality.

Results: Mortality results for each study are included in the following tables:

Table 5. Mortality results during a target animal safety study evaluating chloramine-T treatment of rainbow trout fry (Study No. 1) at 8 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	1	0.3
20 (1X)	0	0	0	0
60 (3X)	0	0	0	0
100 (5X)	5	1.7	8	2.7

Table 6. Mortality results during a target animal safety study evaluating chloramine-T treatment of rainbow trout fry (Study No. 2) at 14 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	7	2.3
20 (1X)	0	0	0	0
60 (3X)	0	0	1	0.3
100 (5X)	7	2.3	10	3.3

Table 7. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 3) at 8 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	0	0
20 (1X)	0	0	5	4.2
60 (3X)	15	12.6	19	16.2
100 (5X)	121	100	121	100

Table 8. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 4) at 14 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	0	0
20 (1X)	0	0	0	0
60 (3X)	21	23.3	21	23.3
100 (5X)	90	100	90	100

Table 9. Mortality results during a target animal safety study evaluating chloramine-T treatment of rainbow trout fingerlings (Study No. 5) at 8 °C. Treatments were given once daily every other day in triplicate, three hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	1	0.7	4	2.7
20 (1X)	0	0	1	0.7
40 (2X)	0	0	0	0
60 (3X)	0	0	0	0

Table 10. Mortality results during a target animal safety study evaluating chloramine-T treatment of fingerling rainbow trout (Study No. 6) at 14 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	0	0
20 (1X)	0	0	0	0
30 (1.5X)	0	0	4	2.7
40 (2X)	0	0	1	0.7
50 (2.5X)	0	0	0	0
60 (3X)	0	0	0	0

Table 11. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 8) at 14 °C. Treatments were given once daily every other day in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	1	2.2	2	4.4
50 (2.5X)	1	2.2	1	2.2
60 (3X)	2	4.4	4	8.9
70 (3.5X)	6	13.3	6	13.3
80 (4X)	17	37.8	17	37.8
100 (5X)	44	97.8	44	97.8

Table 12. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 10) at 14 °C. Treatments were given once daily on consecutive days in triplicate, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	0	0
20 (1X)	0	0	0	0
40 (2X)	0	0	0	0
60 (3X)	0	0	0	0
80 (4X)	30	33.3	31	34.4
100 (5X)	80	88.8	81	90.0

No clinically relevant differences were seen in the mortality rates of any life stage of fish when comparing treatments at 8 °C and 14 °C. In all the studies with statistically significant mortality, the greatest mortality occurred during and after the first treatment, with the signs of toxicity or mortality starting 2 to 2.5 hours into the 3-hour treatment. Toxicity was observed in the fry and fingerling stages at similar dose levels. Greater mortality was seen during some of the studies involving the juvenile life stage.

Conclusions: These studies provide data to support the safety of chloramine-T administered to rainbow trout as a 60-minute bath for 3 consecutive days or 3 treatments on alternate days at a concentration of 20 mg/L.

B. Target Animal Safety Study in Lake Trout

Title: "The Safety of Chloramine-T Use on Lake Trout, *Salvelinus namaycush*"

Study Director: James D. Bowker, MS

Study Location: US Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

General Design of the Study:

a. Purpose: To demonstrate that the proposed maximum effective dosage of chloramine-T (20 mg/L for 1 h) is safe to lake trout.

b. Animals: 240 lake trout fingerlings

c. Test article/controls: Chloramine-T. The control fish were unmedicated.

d. Study Design: Fish were randomly transferred into each of 12 test tanks (20 fish/tank). Chloramine-T was administered in static baths in multiples of 20 mg/L concentrations. Treatments were administered daily for 3 hours each day for 3 treatments. Each dose was tested in duplicate. Approximately 2 hours into the first treatment water samples were collected for analysis of chloramine-T concentration. Mortality observations were made every 30 minutes during each 3-hour treatment.

e. Variables Measured: Mortality and chloramine-T concentrations.

Results: Mortality results are included in the following table.

Table 13. Mortality results during a target animal safety study evaluating chloramine-T treatment of lake trout fingerlings at approximately 12 °C. Treatments were administered once daily for three days, three hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Mortality			
	24 Hours Post-Treatment		14 Days Post-Treatment	
	Number	Percent	Number	Percent
0 (0X)	0	0	0	0
50 (2.5X)	0	0	0	0
100 (5X)	0	0	1	2.9
150 (7.5X)	3	8.8	3	8.8
200 (10X)	27	79.4	27	79.4
300 (15X)	34	100.0	34	100.0

Conclusions: This study provides data to support the safety of chloramine-T administered to lake trout as a 60-minute bath for 3 consecutive days at a concentration of 20 mg/L.

C. Safety Study Evaluating Histopathologic Data

Title: "The Safety of Chloramine-T to Various Life Stages of Rainbow Trout, *Oncorhynchus mykiss*"

Study Director: James D. Bowker, MS

Study Location: US Fish and Wildlife Service
Bozeman Fish Technology Center
Bozeman, MT

General Design of the Study:

a. Purpose: To describe and evaluate the histological effects of six exposure concentrations of chloramine-T (0, 20, 40, 60, 80, and 100 mg/L) on three external (gill, skin, and eye) and two internal (kidney and liver) tissues of juvenile rainbow trout.

b. Animals: 540 rainbow trout fingerlings

c. Test article/controls: Chloramine-T. The control fish were unmedicated.

d. Study Design: Eighteen aluminum tanks with a total water volume of 2.96 ft^3 and a rearing volume of 2.56 ft^3 were used. The tanks were covered with plastic-mesh screen to prevent fish from jumping out of the tanks. Warm and cold-spring water was routed through a head box, where water flow and temperature were adjusted. Water flow to individual tanks was also controlled at spigots for each tank. The water temperature was maintained at ~14°C. Water chemistry data, hardness, alkalinity, and pH were measured on Days 9 and 24. Fish were hand-fed Rangen #4 Custom Trout Grower daily at 1.8% of the estimated mean body weight. Fish were not fed on treatment days. The daily ration was reduced appropriately to account for mortalities and fish removed for histologic sampling. The test tanks were physically separated into one group of 12 tanks and one group of 6 tanks. On Day 1 blinded study participants randomly allocated reference population fish to the 18 test tanks. Groups of 10 fish were transferred to test tanks in three rounds until all tanks contained 30 fish. During the pre-treatment period (Days 1 to 7), fish which were not healthy were replaced with fish from the reference population. On Days 4 and 5, 60 fish were collected from the reference population for pre-exposure health examinations. Fish were measured and visually examined for signs of gross pathology. Samples of gill, skin, eye, kidney, and liver were collected from 10 of the 60 fish collected for pre-exposure examination and from each fish undergoing necropsy during and after the treatment period. A

sample of posterior kidney from 12 fish was inoculated on brain heart infusion agar, maintained at room temperature and evaluated 2 to 7 days later for growth of gram-negative bacteria. A skin scrape and gill squash, from each of 12 fish, were examined for bacteria and parasites. Tanks were checked for mortality twice daily. On Day 3 a non-blinded study participant assigned treatments to the test tanks using a randomized block design. Each chloramine-T dose was tested in triplicate with one replicate of each treatment dose per block.

On Days 8, 10, and 12, chloramine-T treatments were administered. Chloramine-T treatments were administered as a static bath. The water in each tank was stirred to ensure thorough mixing. Approximately 1 hour into each treatment period water samples were collected from each treatment tank for dose verification. Mortality and behavior observations were made every 30 minutes during each 3-hour treatment. A maximum of five moribund fish per tank were collected for gross examination and tissues collection for histopathologic examination on Days 8 and 10. "Healthy-appearing" fish were collected for histologic sampling on Days 12, 19, and 26. Five fish per tank were collected on Day 12 and 2 fish per tank were collected on Days 19 and 26.

A masked histologist examined all tissues. For each histologic criterion evaluated, the histologist ranked the observed changes as 1=none, 2=mild, 3=moderate and 4=severe.

Table 14. Abnormalities found during histologic examination of various tissues.

Gill	Scattered fusion of gill lamellae		Epithelial necrosis	
	Aneurysms in gill capillaries		Basal hyperplasia of gill epithelium	
	Necrotic red blood cells in gill capillaries		Hypertrophy of gill epithelium	
	Epithelial separation from basement membrane			
Kidney	Tubule necrosis		Hydropic degeneration	
	Hyaline droplet degeneration		Tubule swelling	
	Erythrophagia		Hematopoietic hyperplasia	
Eye	Epithelial thickness	Degeneration	Edema	
Liver	Diffuse necrosis	Focal necrosis	Nuclear vacuolation	Cellular vacuolation
Skin	Mucus cell number	Mucus production	Necrosis	Degeneration

The histologist considered moderate and severe changes pathologic or abnormal and provided a report describing his findings.

e. Variables Measured: Mortality, histopathology criteria, behavior, chloramine-T concentration, and water quality parameters.

Results: Total mortality was calculated by adding the number of moribund fish collected to the number of dead fish found and removed from the test tanks. All the fish in the control, 1X, and 2X dose groups appeared healthy throughout the study and the total mortality for these groups was 0%. Moribund fish were observed in the 3X, 4X, and 5X dose groups and the total mortality was 4.4, 60.0, and 98.8% for the groups, respectively. Most of the mortality was observed within 20 to 24 hours after the first chloramine-T treatment. Moderate gill pathologies included epithelial separation of the basement membrane, hypertrophy of the gill epithelium and scattered fusion of gill lamellae, were likely acute effects of treatment with chloramine-T. Erythrophagia of the kidney was considered to be a delayed response by the kidney to attempt to "clean up" some of the gill damage. In the 3X, 4X, and 5X dose groups, pale gills were seen in 22 of 34 moribund fish examined. Pale gills were an indication of deteriorating fish health and were symptomatic of the moderate and severe gill pathologies detected during histological examinations. All the moribund fish examined from the 3X, 4X, and 5X dose groups had one or more moderate or severe pathologies of the gill tissue, kidney tissue, or liver tissue. The gill pathologies were likely acute effects of chloramine-T treatment. The kidney pathologies were likely delayed responses to the gill damage. The focal liver necrosis was judged to be a delayed response to the chloramine-T treatments because the necrosis was seen after the second treatment. The healthy fish collected from the 4X and 5X dose groups had moderate or severe pathologies similar to the pathologies observed in the moribund fish. However, during the post-treatment period, the relative frequency and severity of the pathologies tended to decrease in the gills and to increase in the kidney. The fish in the 3X, 4X, and 5X dose groups that survived the first two treatments were recovering from the toxic effects of the treatments.

The individual measurements of chloramine-T concentrations were between -12.7% and 11.2% of the target concentrations. The mean measured chloramine-T concentrations were between -2.5 and 4.7% of the target concentrations.

The water temperature in the test tanks averaged 14.2 °C and the DO concentration averaged 7.7 mg/L. The measured environmental variables were maintained within ranges suitable for rearing salmonids. During chloramine-T treatments, water temperature and dissolved oxygen concentrations remained within ranges suitable for rearing salmonids.

Conclusions: 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 14 °C. For juvenile rainbow trout reared at a water temperature of ~14 °C, the margin of safety for exposure to chloramine-T extends to at least 40 mg/L. Juvenile rainbow trout are probably most susceptible to the toxic effects of relatively high concentrations of chloramine-T (i.e., $\geq$ 60 mg/L) the first time they are exposed to it. Juvenile rainbow trout that survive the first exposure to relatively high concentrations of chloramine-T (i.e., $\geq$ 60 mg/L) are capable of recovering from the toxic effects of such exposure.