Date of Approval: April 30, 2014

FREEDOM OF INFORMATION SUMMARY

ORIGINAL NEW ANIMAL DRUG APPLICATION

NADA 141-423

HALAMID Aqua

Chloramine-T

Powder for immersion

Freshwater-reared salmonids, walleye, and freshwater-reared warmwater finfish

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

For the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*

For the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*

Sponsored by:

Axcentive SARL

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I. GENERAL INFORMATION

A. File Number

NADA 141-423

B. Sponsor

Axcentive SARL
Chemin de Champouse
Quartier Violesi
13320 Bouc Bel Air
France

Drug Labeler Code: 086009

U.S. Agent for Axcentive SARL:
Rosalie A. Schnick, Principal
Roz Schnick Consulting, LLC
La Crosse, WI

C. Proprietary Name

HALAMID Aqua

D. Established Name

Chloramine-T powder for immersion

E. Pharmacological Category

External disinfectant

F. Dosage Form

Powder

G. Amount of Active Ingredient

98.0 to 100.0% pure

H. How Supplied

5 and 25 kg round plastic containers

I. Dispensing Status

Over-the-counter (OTC)

J. Dosage Regimen

Freshwater-reared salmonids:
12 to 20 milligrams per liter water in a continuous flow water supply or as a static bath once per day for 60 minutes on consecutive or alternative days for three treatments

Walleye:
10 to 20 milligrams per liter water in a continuous flow water supply or as a static bath once per day for 60 minutes on consecutive or alternative days for three treatments

Freshwater-reared warmwater finfish:
20 milligrams per liter water in a continuous flow water supply or as a static bath once per day for 60 minutes on consecutive or alternative days for three treatments

K. Route of Administration

Immersion

L. Species/Class

Freshwater-reared salmonids, walleye, freshwater-reared warmwater finfish

M. Indications

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

For the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*

For the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*

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, 009321, and 010974 which were compiled and submitted 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. A 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.

Similarly, initial doses for the treatment of external columnaris disease in walleye and freshwater-reared warmwater finfish were selected based on published literature and the experience of managers of state and federal fish hatcheries. A dose range of 10 to 20 mg/L administered as a static bath every other day for three treatments was determined to be effective in pilot testing in walleye. Dose confirmation studies at the lower and upper end of the dose range were conducted as described below. A dose of 20 mg/L administered as a static bath every other day for three treatments was determined to be effective in pilot testing for freshwater-reared warmwater finfish. Dose confirmation studies at 20 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 clinical field trials for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp. and for the control of mortality in walleye and freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*. Additionally, a special study to measure concentrations of the drug in a flow-through system was conducted in a common concrete raceway.

1. Clinical Effectiveness Field Study

   a. Title: "Efficacy of Chloramine-T to Control Mortality Caused by Bacterial Gill Disease (BGD) in Fingerling Rainbow Trout." Study 4000-1-04 (July 1998)

   b. Study Director: James D. Bowker
   U.S. Fish & Wildlife Service
   Aquatic Animal Drug Approval Partnership Program
   Bozeman, MT

   Study Investigator: David Oviedo
   US Fish and Wildlife Service
   Uvalde, TX

   Study Location: Hotchkiss National Fish Hatchery
   Hotchkiss, CO

   c. Study Design:

      1) Objective: To evaluate the effectiveness of chloramine-T as a bath at a concentration of 12 mg/L for 60 minutes every other day for three

treatments for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in rainbow trout.

2) Study Animals: Approximately 6000 fingerling rainbow trout
Mean body weight 2.66 g, Mean length 6.1 cm

3) Test article/Controls: Test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was initiated at the beginning of a disease outbreak. The fish were randomly transferred into each of six test tanks (1000 fish/tank). Fish were evaluated pre-treatment for clinical signs of the disease by removing five fish from each test tank. The body surface, fins, gills, and internal organs were examined. Kidney inocula were streaked on tryptone yeast extract with salts and brain heart infusion media to rule-out the presence of systemic bacteria. BGD was confirmed by preparing stained gill squash slides and identifying the etiologic agent. Unmedicated control and chloramine-T treatment groups were tested in triplicate experimental units (test tanks). Sixty-minute chloramine-T treatments of 12 mg/L were administered as a static bath every other day for three days. The duration of the treatment period was five days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 19 days.

5) Variables Measured: Cumulative mortality was the primary variable and was evaluated twice daily. Chloramine-T concentrations in the test tanks were measured using a colorimetric method. Temperature and dissolved oxygen were measured twice daily. Water hardness and pH were measured at the beginning and at termination of the study.

d. Statistical Analysis: Mortality rates were analyzed using a generalized linear model with a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included. The results were evaluated at a significance level of α=0.05.

e. Results: There was a statistically significant difference ($p<0.0001$) between the treated and control groups with a lower cumulative mortality observed in the treated group. Mortality results are summarized in the table below.

Table 1. Mortality results for an effectiveness study in fingerling rainbow trout treated on three alternate days followed by a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	773/3000	25.8%
12 mg/L	171/3000	5.7%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 12 mg/L. 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 (as $CaCO_3$) and pH values were approximately 396 mg/L and 7.8, respectively.

f. Conclusion: This study demonstrates the effectiveness of chloramine-T administered at a concentration of 12 mg/L for 60 minutes on three alternate days for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in rainbow trout.

2. Clinical Effectiveness Field Trial

a. Title: "Efficacy of Chloramine-T to Control Mortality in Apache Trout (*Oncorhynchus apache*) Caused by Bacterial Gill Disease Associated with Flavobacters." Study 4000-1-003 (June 1997 to July 1997)

b. Study Director: James D. Bowker
U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Bob David
US Fish and Wildlife Service
Whiteriver, AZ

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

c. Study Design:

1) Objective: 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 three treatments for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in apache trout.

2) Test Animals: Approximately 14,040 fingerling apache trout
Mean body weight 0.9 g, Mean length 4.9 cm

3) Test article/Controls: Test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was initiated at the beginning of a disease outbreak. The fish were randomly transferred into each of six test tanks (approximately 2340 fish/tank). Fish were evaluated pre-treatment for clinical signs of the disease by removing six fish from each test tank. The body surface, fins, gills, and internal organs were examined. Kidney inocula were streaked on tryptose soy agar media to rule-out the presence of systemic bacteria. BGD was confirmed by preparing stained gill squash slides and identifying the etiologic agent. Untreated control and chloramine-T treatment groups were tested in triplicate experimental units (test tanks). Sixty-minute chloramine-T treatments of 12 mg/L were administered as a static bath every other day for three days. The duration of the treatment period was five days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 19 days.

5) Variables Measured: Cumulative mortality was the primary variable and was evaluated twice daily. Chloramine-T concentrations were measured using a colorimetric method. Temperature and dissolved oxygen were measured twice daily. Water hardness and pH were measured at the beginning and at termination of the study.

d. Statistical Analysis: Mortality rates were analyzed using a generalized linear model with a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included. The results were evaluated at a significance level of α=0.05.

e. Results: There was a statistically significant difference between the treated and control groups ($p<0.0001$) with a lower cumulative mortality observed in the treated group. Mortality results are summarized in the table below.

Table 2. Mortality results for an effectiveness study in fingerling apache trout treated on three alternate days followed by a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	6397/6524	98.1%
12 mg/L	2508/6446	38.9%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 12 mg/L. Temperature and 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 (as $CaCO_3$) and pH values were approximately 45 mg/L and 7.1, respectively.

f. Conclusion: This study demonstrates the effectiveness of chloramine-T administered at a concentration of 12 mg/L for 60 minutes on three alternate days for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in apache trout.

3. Clinical Effectiveness Field Study

a. Title: "Efficacy of Chloramine-T to Control Mortality Caused by Bacterial Gill Disease Associated with Flavobacters in Fall Chum Salmon (*Oncorhynchus keta*)." Study 4000-1-002 (May 1997 to June 1997)

Study Director: James D. Bowker
U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Larry Telles
US Fish and Wildlife Service
Quilcene, WA

Study Location: Quilcene National Fish Hatchery
Quilcene, WA

b. Study Design:

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

2) Test Animals: Approximately 96,594 fall chum salmon
Mean body weight 0.95 g, Mean length 4.8 cm

3) Test article/Controls: Test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was initiated at the beginning of a disease outbreak. The fish were randomly transferred into each of six test tanks (approximately 13,419 to 19,248 fish/tank). Fish were evaluated pre-treatment for clinical signs of the disease by removing five fish from each test tank. The body surface, fins, gills, and internal organs were examined, gill tissues were preserved in Davidson's solution for histological examination. Kidney inocula were streaked on tryptone yeast extract with salts and brain heart infusion media to rule-out the presence of systemic bacteria. BGD was confirmed by preparing stained gill squash slides and identifying the etiologic agent. Untreated control and chloramine-T treatment groups were tested in triplicate experimental units (test tanks). Sixty-minute chloramine-T treatments of 12 mg/L were administered as a static bath every other day for three days. The duration of the treatment period was five days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 19 days.

5) Variables Measured: Cumulative mortality was the primary variable and was evaluated twice daily. Chloramine-T concentrations in the test tanks were measured using a colorimetric method. Temperature and dissolved oxygen were measured twice daily. Water hardness and pH were measured at the beginning and at termination of the study.

c. Statistical Analysis: Mortality rates were analyzed using a generalized linear model with a binomial error distribution and a logit link function. Treatment was included as a fixed effect and an overdispersion parameter was included. The results were evaluated at a significance level of α=0.05.

d. Results: There was a statistically significant difference between the treated and control groups ($p<0.0001$) with a lower cumulative mortality observed in the treated group. Mortality results are summarized in the table below.

Table 3. Mortality results for an effectiveness study in fall chum salmon treated on three alternate days followed by a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	48,067/48,241	99.6%
12 mg/L	3907/48,218	8.1%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 12 mg/L. Temperature and 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 (as $CaCO_3$) and pH values were approximately 38 mg/L and 7.3, respectively.

e. Conclusion: This study demonstrates the effectiveness of chloramine-T administered at a concentration of 12 mg/L for 60 minutes on three alternate days for the control of mortality due to bacterial gill disease associated with *Flavobacterium* spp. in fall chum salmon.

4. Clinical Field Effectiveness Study

a. Title: "Efficacy of Chloramine-T to Control Mortality Associated with External Columnaris on Walleye (*Sander vitreus*) Fingerlings." Study CAP-02-CLT-06 (July 2002)

Study Investigator: Jeffrey J. Rach
US Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

Study Location: Rathbun Fish Hatchery
Moravia, IA

b. Study Design:

1) Objective: To evaluate the effectiveness of chloramine-T as a static bath at concentrations of 0, 10, and 20 mg/L for 60 minutes every other day for three treatments to control mortality due to external columnaris disease associated with *Flavobacterium columnare* on walleye fingerlings.

2) Test Animals: 306 fingerling walleye
Mean body weight 12 g, Length 10-13 cm

3) Test article/Controls: The test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was conducted with naturally infected walleye. A total of 306 fingerlings from the reference population were randomly assigned to nine tanks (34/tank). The study had a completely randomized design with three treatment groups and three replicates of each treatment. Three samples of four fish each were collected from the reference population for disease diagnosis. Fish with yellow, mucoid lesions predominately on the tail and in the areas of the dorsal, pelvic, and pectoral fins were selected. Disease diagnosis was made by observation of distinct strands or "haystacks" of bacteria on wet mounts and stained samples from skin scrapes and gill smears. Sixty minute chloramine-T treatments of 10 mg/L and 20 mg/L were administered as a static bath every other day for three treatments. Tanks of infected, unmedicated fish served as controls (0 mg/L). The duration of the

treatment period was five days and was followed by a post-treatment observation period of 10 days resulting in a total study duration of 15 days. Fish were fed a standard commercial walleye grower feed. Fish were not fed on treatment days. Water was supplied to the tanks from a rearing tank by using a submersible pump. Water flow rate to the test tanks was checked and adjusted daily.

5) Variables Measured: Mortality was the primary variable and was recorded daily with the first count made 24 hours after the first treatment. Water samples were collected from one replicate of each treatment group during each of the three treatments. Water was analyzed for chloramine-T concentration. Dissolved oxygen, water hardness, total alkalinity, temperature, and pH were recorded daily for each test tank during the treatment period.

c. Statistical Analysis: Mortality rates were analyzed using a nonlinear mixed effects model with a binomial error distribution and logit link function. Treatment was included as a fixed effect treatment, tank as a random effect and an overdispersion parameter. The results were evaluated at a significance level of α=0.05.

d. Results: There was a statistically significant difference when each of the treated groups is compared to control (p = 0.022 for 0 vs 10 mg/L and p=0.044 for 0 vs 20 mg/L) with lower cumulative mortality observed in the treated groups. Mortality results are summarized in the table below.

Table 4. Mortality results for an effectiveness study in fingerling walleye treated on three alternate days followed by a 10-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	90/103	87.4%
10 mg/L	44/102	43.1%
20 mg/L	67/102	65.7%

In all groups, mortality was greatest during the treatment period. In two of the control tanks, all of the fish died by Day 4. In the 10 mg/L group mortality was higher in one tank (28 fish) than the other two tanks (6 and 10 fish).

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve concentrations of 10 and 20 mg/L. The average water temperature was 25.2 °C. The total hardness (as CaCO3) and alkalinity of the water were 90 mg/L and 75 mg/L, respectively. The average pH and dissolved oxygen of the water were 7.58 and 7.4 mg/L, respectively.

e. Conclusion: This study demonstrates the effectiveness of chloramine-T administered at concentrations of 10 mg/L and 20 mg/L for 60 minutes on three alternate days for three treatments for the control of mortality due to external columnaris disease associated with *Flavobacterium columnare* in walleye.

5. Clinical Field Effectiveness Study

a. Title: "The Efficacy of Chloramine-T to Control Mortality of Walleye *Sander vitreus* Caused by Columnaris, Causative Agent *Flavobacterium columnare*." Study CHLT-96-EFF-07 (June 2006)

b. Study Director: James D. Bowker
U.S. Fish & Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Alan Johnson
Rathbun Fish Culture and Fisheries Research Facility
Moravia, IA

Study Location: Iowa Department of Natural Resources
Rathbun Fish Culture and Fisheries Research Facility
Moravia, IA

c. Study Design:

1) Objective: To evaluate the effectiveness of chloramine-T administered at 20 mg/L in a static bath for 60 minutes on alternate days for three treatments to control mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare.*

2) Test Animals: 1,736 fingerling walleye (*Sander vitreus*)
Mean body weight 0.83 g, Mean length 4.6 cm

3) Test article/controls: The test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was conducted with naturally infected walleye. One thousand seven hundred thirty six (1,736) walleye from the reference population were randomly allocated into each of eight rectangular fiberglass test tanks (217/tank). Five fish from the reference population were evaluated pre-treatment for clinical signs of disease. The body surface was examined and external columnaris disease was confirmed by preparing and identifying the etiologic agent using microscopic examination of wet mounts of skin scrapes to detect the presence of the typical "haystacks" of bacteria associated with external columnaris infection. One moribund or recently dead fish was sampled from three treated and one control tank at the end of the treatment period to reconfirm disease diagnosis. Microscopic wet mounts confirmed the initial disease diagnosis of external columnaris disease. The tanks were randomly assigned to treatments according to a randomized complete block design. There were two treated tanks and two control tanks in each of two blocks. Tanks of infected, unmedicated fish served as controls (0 mg/L). Water inflow to each test tank was set at 2 L/min. The duration of the treatment period was five days and was followed by a post-treatment observation period of 11 days resulting in a total study duration of 16 days. Fish were fed a commercial walleye feed. Fish were not fed on treatment days. No concomitant therapies were administered to the test fish during the study.

5) Variables measured: Cumulative mortality was the primary variable and was recorded daily starting on the first day of the treatment period. Chloramine-T concentrations in the test tanks were measured using a colorimetric method. Temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity, and pH were measured twice daily.

d. Statistical Analysis: Mortality rates were analyzed using a generalized linear mixed model using a binomial error distribution and logit link function with treatment as a fixed effect and block as a random effect. The model included an overdispersion parameter and utilized the Kenward-Rogers adjustment for degrees of freedom. The analysis was performed at post-treatment Day 5 and the primary time point of post-treatment Day 11. The results were evaluated at a significance level of α=0.05.

e. Results: There was a statistically significant difference between the treated and control groups on post-treatment Day 5 (p= 0.0432) but not between the two groups on post-treatment Day 11 (p=0.1859). Mortality rates were lower on each day in the treated group than the control group. Mortality results are summarized in the table below.

Table 5. Mortality results for an effectiveness study in fingerling walleye treated on three alternate days at Day 5 and Day 11 post-treatment.

Chloramine-T concentration	Cumulative Mortality on day 5	Percent Mortality on day 5	Cumulative Mortality on day 11	Percent Mortality on day 11
0 mg/L	713/868	82.1%	721/868	83.1%
20 mg/L	659/868	75.9%	686/868	79.0%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 20 mg/L. Mean hardness, alkalinity, and pH were 154 mg/L $CaCO_3$, 110 mg/L $CaCO_3$, and 7.7, respectively. Water temperatures ranged from 20.5 to 25.5 ^{o}C. Dissolved oxygen concentrations ranged from 5.7 to 9.6 mg/L.

f. Conclusions: Treatment of this outbreak began late in the disease progression, which resulted in higher than anticipated mortality in all groups. This study supports the effectiveness of chloramine-T administered at concentrations of 20 mg/L for 60 minutes on three alternate days for three treatments for the control of mortality due to external columnaris disease associated with *Flavobacterium columnare* in walleye.

6. Clinical Field Effectiveness Study

a. Title: "The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare*." Study CHLT-07-EFF.1-07 (August 2008)

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Michael Matthews
Florida Bass Conservation Commission
Richloam Fish Hatchery
Webster, FL

Study Location: Richloam Fish Hatchery
Webster, FL

b. Study Design:

1) Objective: To evaluate the effectiveness of chloramine-T as a static bath at a concentration of 20 mg/L for 60 minutes per day on three alternate days to control mortality in fingerling largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.

2) Test Animals: 2,098 fingerling largemouth bass *Micropterus salmoides*
Mean body weight 17.65 g, mean length 12.48 cm

3) Test Article/Controls: The test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was conducted with naturally infected largemouth bass. A total of 2,098 fingerlings from the reference population were randomly assigned to eight tanks (approximately 262 per tank). The study had a completely randomized design with four treatment tanks and four control tanks. Ten fish were collected from the reference population for disease diagnosis. All external and internal features appeared normal with the exception of skin lesions ("saddleback" and lesions on fish body surface and tail) characteristic of external columnaris disease. Disease diagnosis was made by observation of distinct strands or "haystacks" of bacteria on wet mounts and stained samples from skin scrapes. Sixty-minute chloramine-T treatments of 20 mg/L were administered as a static bath once per day on three alternate days. Fish in the control tanks were unmedicated (0 mg/L). The treatment period was five days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 19 days. Fish were fed feed specifically formulated for largemouth bass. Water flow rate to the test tanks was checked and adjusted daily.

5) Measurements and Observations: Mortality was the primary variable and was recorded twice daily. Water samples were analyzed for chloramine-T concentration. Water temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity, and pH were measured three times during the study.

The effectiveness of chloramine-T to control mortality in largemouth bass due to external columnaris disease was evaluated by comparing the proportion of mortalities in the chloramine-T treatment groups to the untreated control groups.

c. Statistical Analysis: Mortality rates were analyzed using a generalized linear mixed model with a binomial error distribution and logit link function with

treatment as a fixed effect and block as a random effect. The model included an overdispersion parameter and utilized the Kenward-Rogers adjustment for degrees of freedom. The results were evaluated at a significance level of α=0.05.

d. Results: There was a statistically significant difference between the treated and control groups (p=0.0032) with a lower cumulative mortality observed in the treated group 14 days post-treatment. Mortality results are summarized in the table below.

Table 6. Mortality results for an effectiveness study in largemouth bass treated on three alternate days followed by a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	646/1032	62.6%
20 mg/L	487/1066	45.7%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 20 mg/L. The average water temperature was 24.8 °C. The total hardness and alkalinity of the water (as $CaCO_3$) were 320 mg/L and 345 mg/L, respectively. The average pH and dissolved oxygen of the water were 7.6 and 13.8 mg/L, respectively.

e. Conclusions: This study demonstrates the effectiveness of chloramine-T administered at 20 mg/L for 60 minutes per day on three alternate days for the control of mortality due to external columnaris disease associated with *Flavobacterium columnare* in largemouth bass *Micropterus salmoides.*

7. Clinical Field Effectiveness Study

a. Title: "The Efficacy of Chloramine-T to Control Mortality of Largemouth Bass *Micropterus salmoides* Caused by External Columnaris, Causative Agent *Flavobacterium columnare.*" Study CHLT-07-EFF.1-01 (June 2007 to July 2007)

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Michael Matthews
Florida Bass Conservation Commission
Richloam Fish Hatchery
Webster, FL

Study Location: Richloam Fish Hatchery
Webster, FL

b. Study Design:

1) Objective: To evaluate the effectiveness of chloramine-T as a static bath at a concentration of 20 mg/L for 60 minutes every day for three consecutive days to control mortality in fingerling largemouth bass due to external columnaris disease associated with *Flavobacterium columnare*.

2) Test Animals: 3,630 fingerling largemouth bass
Mean body weight 4.7 g, length 6.9 cm

3) Test Article/Controls: The test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was conducted with naturally infected largemouth bass. A total of 3,630 fingerlings were randomly assigned to twelve tanks (approximately 300/tank) from the reference population. The study had a completely randomized design with six treatment tanks and six control tanks. Ten fish were collected from the reference population for disease diagnosis. All external and internal features appeared normal with the exception of skin lesions ("saddleback" and lesions on fish body surface and tail) characteristic of external columnaris disease. Disease diagnosis was made by observation of distinct strands or "haystacks" of bacteria on wet mounts and stained samples from skin scrapes. Sixty-minute chloramine-T treatments of 20 mg/L were administered as a static bath daily for three treatments. Tanks of infected, unmedicated fish served as controls (0 mg/L). The treatment period was three days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 17 days. Fish were fed feed specifically formulated for bass. Water flow rate to the test tanks was checked and adjusted daily.

5) Measurements and Observations: Mortality was the primary variable and was recorded daily. Water samples were collected from all test tanks during each of the three treatments. Water was analyzed for chloramine-T concentration. Water temperature and dissolved oxygen were measured twice daily. Water hardness, alkalinity and pH were also measured.

The effectiveness of chloramine-T immersion to control mortality in largemouth bass due to external columnaris disease was evaluated by comparing the proportion of mortalities in the chloramine-T treatment groups to the untreated control groups.

c. Statistical Analysis: Mortality rates were analyzed using a generalized linear mixed model using a binomial error distribution and logit link function, with treatment as a fixed effect and block as a random effect. The model included an overdispersion parameter and utilized the Kenward-Rogers adjustment for degrees of freedom. The results were evaluated at a significance level of α=0.05.

d. Results: There was a statistically significant difference between the treated and control groups (p=0.010) with a lower cumulative mortality observed in the treated group 14 days post-treatment. Mortality results are summarized in the table below.

Table 7. Mortality results for a field effectiveness study in largemouth bass with a 3-day treatment period and a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	642/1817	35.3%
12 mg/L	487/1813	26.9%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 20 mg/L. The average water temperature was 25.2 °C. The total hardness (as $CaCO_3$) and alkalinity of the water were 245 mg/L and 373 mg/L, respectively. The average pH and dissolved oxygen of the water were 8.05 and 15.8 mg/L, respectively.

e. Conclusions: This study demonstrates the effectiveness of chloramine-T administered at 20 mg/L for 60 minutes per day for three consecutive days for the control of mortality due to external columnaris disease associated with *Flavobacterium columnare* in largemouth bass.

8. Clinical Field Effectiveness Study

a. Title: "The Efficacy of Chloramine-T to Control Mortality of Bluegill *Lepomis macrochirus* Caused by External Columnaris, Causative Agent *Flavobacterium columnare.*" Study CHLT-07-EFF.1-05 (April 2008)

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

Study Investigator: Michael Matthews
Florida Bass Conservation Commission
Richloam Fish Hatchery
Webster, FL

Study Location: Richloam Fish Hatchery
Webster, FL

b. Study Design:

1) Objective: To evaluate the effectiveness of chloramine-T as a static bath at a concentration of 20 mg/L for 60 minutes per day on three alternate days to control mortality in fingerling bluegill due to external columnaris disease associated with *Flavobacterium columnare*.

2) Test Animals: 1,465 fingerling bluegill *Lepomis macrochirus*
Mean body weight 15.8 g, Mean length 8.8 cm

3) Test Article/Controls: The test article was chloramine-T. The control fish were unmedicated.

4) Experimental Design: The study was conducted with naturally infected bluegill. A total of 1,465 fingerlings were randomly assigned to eight tanks (approximately 200 per tank) from the reference population. The study had a completely randomized design with four treatment tanks and four control tanks. Ten fish were collected from the reference population for disease diagnosis. All external and internal features appeared normal with the exception of skin lesions ("saddleback" and lesions on fish body surface and tail) characteristic of external columnaris disease. Disease diagnosis was made by observation of distinct strands or "haystacks" of bacteria on wet mounts and stained samples from skin scrapes. Fish were fed feed specifically formulated for bluegill. Sixty-minute chloramine-T treatments of 20 mg/L were administered as a static bath once per day on three alternate days. Fish in the control tanks were unmedicated (0 mg/L). The treatment period was five days and was followed by a post-treatment observation period of 14 days resulting in a total study duration of 19 days. Water flow rate to the test tanks was checked and adjusted daily.

5) Measurements and Observations: Mortality was the primary variable and was recorded once daily. Four water samples were collected from test tanks and two samples were collected from control tanks. Water was analyzed for chloramine-T concentration. Water temperature and dissolved oxygen were measured once daily. Water hardness, alkalinity and pH were measured three times during the study.

   The effectiveness of chloramine-T immersion to control mortality in bluegill due to external columnaris disease was evaluated by comparing the proportion of mortalities in the chloramine-T treatment groups to the untreated control groups.

c. Statistical Analysis: Mortality rates were analyzed using a generalized linear mixed model using a binomial error distribution and a logit link function, with treatment as a fixed effect and block as a random effect. The model included an overdispersion parameter and utilized the Kenward-Rogers adjustment for degrees of freedom. The results were evaluated at a significance level of α=0.05.

d. Results: There was a statistically significant difference between the treated and control groups (p=0.0304) with a lower cumulative mortality observed in the treated group 14 days post-treatment. Mortality results are summarized in the table below.

Table 8. Mortality results for an effectiveness study in bluegill treated on three alternate days followed by a 14-day post-treatment period.

Chloramine-T Concentration	Cumulative Mortality	Percent Mortality
0 mg/L	199/733	27.1%
12 mg/L	95/732	13.0%

Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve a concentration of 20 mg/L. The

average water temperature was 22.2 °C. The total hardness (as $CaCO_3$) and alkalinity of the water were 317 mg/L and 337 mg/L, respectively. The average pH and dissolved oxygen of the water were 7.59 and 11.9 mg/L, respectively.

e. Conclusions: This study demonstrates the effectiveness of chloramine-T administered at 20 mg/L for 60 minutes per day on three alternate days for the control of mortality due to external columnaris disease associated with *Flavobacterium columnare* in bluegill *Lepomis macrochirus.*

9. 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.

a. Title: "Analytical Verification of Chloramine-T to Confirm Target Dosage in a Bath Solution Administered Using a Flow-Through Treatment Method" (July 2001)

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

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

b. Study Design:

1) Objective: 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.

2) Test Animals: None

3) Test article/Controls: Test article was chloramine-T. There were no control animals used.

4) Experimental 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)

5) Variables Measured: Chloramine-T concentration. 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.

c. 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.

d. 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.

e. Conclusion: This study demonstrates that 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

1. Title: "The Safety of Chloramine-T to Various Life Stages of Rainbow Trout (*Oncorhynchus mykiss*)" Study BFTC-99-CHLT-TAS-(#01-06, 08, 10) (April 1999 to May 1999)

   Study Director: James D. Bowker
   U.S. Fish and Wildlife Service
   Aquatic Animal Drug Approval Partnership Program
   Bozeman, MT

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

2. Study Design:

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

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

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

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

   Table 9. 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 (fish per tank)	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

The same protocol was used for the eight studies. Chloramine-T was administered in static baths in multiples of a 20 mg/L concentration, 3 tanks per treatment 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, 3 hours per treatment. 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

3. 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. 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.

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

Table 10. Mortality results during a target animal safety study evaluating chloramine-T treatment of rainbow trout fry (Study No. 1) at 8 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	0.3 (1)
20 (1X)	0 (0)	0 (0)
60 (3X)	0 (0)	0 (0)
100 (5X)	1.7 (5)	2.7 (8)

Table 11. Mortality results during a target animal safety study evaluating chloramine-T treatment of rainbow trout fry (Study No. 2) at 14 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	2.3 (7)
20 (1X)	0 (0)	0 (0)
60 (3X)	0 (0)	0.3 (1)
100 (5X)	2.3 (7)	3.3 (10)

Table 12. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 3) at 8 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	0 (0)
20 (1X)	0 (0)	4.2 (5)
60 (3X)	12.6 (15)	16.2 (19)
100 (5X)	100 (121)	100 (121)

Table 13. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 4) at 14 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	0 (0)
20 (1X)	0 (0)	0 (0)
60 (3X)	23.3 (21)	23.3 (21)
100 (5X)	100 (90)	100 (90)

Table 14. Mortality results during a target animal safety study evaluating chloramine-T treatment of fingerling rainbow trout (Study No. 5) at 8 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0.7 (1)	2.7 (4)
20 (1X)	0 (0)	0.7 (1)
40 (2X)	0 (0)	0 (0)
60 (3X)	0 (0)	0 (0)

Table 15. Mortality results during a target animal safety study evaluating chloramine-T treatment of fingerling rainbow trout (Study No. 6) at 14 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	0 (0)
20 (1X)	0 (0)	0 (0)
30 (1.5X)	0 (0)	2.7 (4)
40 (2X)	0 (0)	0.7 (1)
50 (2.5X)	0 (0)	0 (0)
60 (3X)	0 (0)	0 (0)

Table 16. Mortality results during a target animal safety study evaluating chloramine-T treatment of juvenile rainbow trout (Study No. 8) at 14 °C.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	2.2 (1)	4.4 (2)
50 (2.5X)	2.2 (1)	2.2 (1)
60 (3X)	4.4 (2)	8.9 (4)
70 (3.5X)	13.3 (6)	13.3 (6)
80 (4X)	37.8 (17)	37.8 (17)
100 (5X)	97.8 (44)	97.8 (44)

Table 17. 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, for three days, 3 hours per treatment.

Chloramine-T Concentration (mg/L)	Cumulative Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
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)	33.3 (30)	34.4 (31)
100 (5X)	88.8 (80)	90 (81)

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 (abnormal behavior and morbidity) 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.

5. Conclusion: These studies demonstrate that there is an adequate margin of safety for chloramine-T when administered to rainbow trout as a 60-minute bath on 3 consecutive or alternate days for 3 treatments at a concentration of 20 mg/L.

B. Target Animal Safety Study in Lake Trout

1. Title: "The Safety of Chloramine-T Use on Lake Trout, *Salvelinus namaycush*" Study BFTC-99-CHLT-TAS-LKT-01 (July 2001 to August 2001)

   Study Director: James D. Bowker
   U.S. Fish and Wildlife Service
   Aquatic Animal Drug Approval Partnership Program
   Bozeman, MT

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

2. Study Design:

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

   b. Animals: 240 lake trout fingerlings
   Mean body weight 6.1 g, Mean length 9.6 cm

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

   d. Experimental 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, 2 tanks per treatment concentration. Treatments were administered daily for 3 hours each day for 3 treatments. 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.

3. Results: Mortality results are included in the following table.

   Table 18. 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 Percent Mortality at 24 hours post-treatment (number of mortalities)	Cumulative Percent Mortality at 14 days post-treatment (number of mortalities)
0 (0X)	0 (0)	0 (0)
50 (2.5X)	0 (0)	0 (0)
100 (5X)	0 (0)	0 (0)
150 (7.5X)	8.8 (3)	8.8 (3)
200 (10X)	79.4 (27)	79.4 (27)
300 (15X)	100 (34)	100 (34)

4. Conclusion: This study supports an adequate margin of safety for 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

1. Title: "The Safety of Chloramine-T to Various Life Stages of Rainbow Trout, *Oncorhynchus mykiss*" (February 2000 to March 2000)

Study Director: James D. Bowker
U.S. Fish and Wildlife Service
Aquatic Animal Drug Approval Partnership Program
Bozeman, MT

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

2. Study Design:

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
Mean body weight 42.0 g, Mean length 15.6 cm

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

d. Experimental 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 six 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 3 tanks 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 one 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 collected. For each histologic criterion evaluated, the histologist ranked the observed changes as 1=none, 2=mild, 3=moderate and 4=severe.

Table 19. Histologic criteria for evaluation of rainbow trout fingerlings.

Tissue	Histologic criteria assessed
Gill	Scattered fusion of gill lamellae, aneurysms in gill capillaries, necrotic red blood cells in gill capillaries, epithelial necrosis, basal hyperplasia of gill epithelium, 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.

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

3. 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.
Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve concentrations of 20, 40, 60, 80, and 100 mg/L.

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.

4. 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., ≥ 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., ≥ 60 mg/L) are capable of recovering from the toxic effects of such exposure.

D. Target Animal Safety of Chloramine-T to Cool- and Warmwater Fish

1. Title: "Toxicity Assessment of Chloramine-T to Cool- and Warmwater Fish" (June 1999 to November 2000)

   Study Director: Mark P. Gaikowski

   U.S. Geological Survey
   Upper Midwest Environmental Sciences Center
   La Crosse, WI

   Study Location: Upper Midwest Environmental Sciences Center
   La Crosse, WI

2. Study Design:

a. Purpose: Three separate experiments were conducted to determine the acute toxicity and histopathological effects of chloramine-T treatment to freshwater-reared cool- and warmwater finfish. The first experiment was designed to evaluate the effect of life stage, temperature, and exposure duration on representative species. The second experiment was designed to evaluate the effect of different water chemistry parameters on a known sensitive species. The third experiment was designed to determine the presence of histopathological lesions in representative species.

b. Test Animals:

Experiment 1: lake sturgeon fry, northern pike fry, walleye fry, channel catfish fry, largemouth bass fry, walleye fingerlings, and channel catfish fingerlings

Experiment 2: walleye fingerlings

Experiment 3: walleye fingerlings and channel catfish fingerlings

c. Treatment Groups:

Experiment 1: 4 treated groups, 1 untreated group, 3 replicates per treatment group, 10 fish per replicate

Experiment 2: 4 treated groups, 1 untreated group, 6 replicates per treatment group, 3 fish per replicate

Experiment 3: 3 treated groups, 1 untreated group, 3 replicates per treatment group, 10 fish per replicate

d. Dosage Form: 96-100% (w/w) chloramine-T

e. Route of Administration: Immersion (bath)

f. Doses Used:

Experiment 1: 0, 20, 60, 100, and 200 mg/L

Experiment 2: 0, 20, 60, 100, and 200 mg/L

Experiment 3: 0, 20, 50, and 80 mg/L

g. Test Duration:

Experiment 1: Exposed once daily for 4 days (60 or 180 minutes) followed by a 4-day post treatment period.

Experiment 2: Exposed once daily for 4 days (60 minutes) followed by a 4-day post treatment period.

Experiment 3: Exposed once daily for 12 days (180 minutes) followed by a 14-day post treatment period.

h. Variables:

Experiment 1: Study cumulative mortality was the primary variable. Daily mortality was recorded for each of the treatment and post treatment days.

Secondary variables included fish weight and length. Data on feeding behavior, general health observations, and water quality were also collected.

Experiment 2: Study cumulative mortality was the primary variable. Daily mortality was recorded for each of the treatment and post treatment days. Secondary variables included water chemistry and fish weight and length. Data on feeding behavior and general health observations were also collected.

Experiment 3: Study cumulative mortality was a primary variable. Daily mortality was recorded for each of the treatment and post treatment days. Frequency of occurrence of histopathologic lesions (eye, skin, kidney, liver, spleen, gills) was also a primary variable. Secondary variables included hematocrit, weight, and length. Data on feeding behavior, general health observations, and water quality were also collected.

i. Methods:

Experiment 1:
Test fish were randomly distributed to each test aquarium. Fish were allowed to acclimate to the test aquaria for 32 to 48 hours before the first exposure. Test article was dissolved in 50 to 100 mL of aquarium water and this solution was added to the aquaria and gently mixed. Chloramine-T concentration was verified with a Hach DPD colorimeter in one water sample removed from each test aquarium between 20 to 30 minutes after the test article was added and mixed. At the initiation of the study, one test aquarium from each treatment concentration was sampled in triplicate to verify analytical precision. Subsequently, this was amended to sampling one aquarium in triplicate from one concentration. Fish were exposed to static chloramine-T bath exposures for 60 or 180 minutes. One exposure system consisting of 15 aquaria (4 concentrations and one untreated control, 3 replicates/concentration) was prepared for each species, exposure duration, exposure temperature, and life stage combination tested. Water quality parameters of temperature, dissolved oxygen, and pH were determined in all aquaria on each exposure day. Flow rates were adjusted daily and recorded.

Mortality was recorded immediately after the initial exposure and before and after exposure for the second, third, and fourth exposures. Following the fourth exposure, mortality was recorded at 24 hour intervals for 96 hours past the fourth exposure. All fish that survived to 96 hours after the last exposure were euthanized by immersion in ice water. Wet weight and total length of all fish were determined after death or euthanasia.

Feeding activity was qualitatively assessed at each feeding using the following scale: 0 - less aggressive / less eaten than controls, 1 - similar to controls, 2 - more aggressive/more eaten than controls. Gross necropsy was performed on all fish that died during the observation period and on two surviving fish from each aquarium with surviving fish at the end of the observation period. Gross necropsy was performed on at least two fish from the acclimation tank before the first exposure to chloramine-T (initial controls) and after the last day of observation (i.e., at the same time that necropsies were performed on fish from test aquaria).

Experiment 2:

Walleye fingerlings were gradually acclimated to reconstituted soft water over 17 days. Test fish were randomly distributed to each test aquarium. Fish were allowed to acclimate to the aquaria for 32 to 48 hours before the first exposure. Test article was dissolved in 50 mL of aquarium water and the solution added to the aquaria and gently mixed. Chloramine-T concentration was verified with a Hach DPD colorimeter in one water sample removed from each of three of the six test aquaria between 20 to 30 minutes after the test article was added and mixed. Sixty minute static chloramine-T bath exposures were administered on Days 0, 1, 2, and 3. One exposure system consisting of 30 aquaria (4 concentrations and one untreated control, 6 replicates/concentration) was prepared. Temperature, dissolved oxygen, and pH were measured. Total hardness and alkalinity of reconstituted soft water used were determined on each day walleye fingerlings were in the 1-L glass aquaria. Flow rates were adjusted daily and recorded.

Mortality was recorded immediately after the initial exposure and before and after exposure for the second, third, and fourth exposures. Following the fourth exposure, mortality was recorded at 24 hour intervals for 96 hours past the fourth exposure. All fish that survived to 96 hours after the last exposure were euthanized by immersion in ice water. Wet weight and total length of all fish were determined after death or euthanasia.

Feeding activity was qualitatively assessed at each feeding using the following scale: 0 - less aggressive/less eaten than controls, 1 - similar to controls, 2 - more aggressive/more eaten than controls. Gross necropsy was performed on all fish that died during the observation period and on two surviving fish from each aquarium with surviving fish at the end of the observation period. Gross necropsy was performed on at least two fish from the acclimation tank before the first exposure to chloramine-T (initial controls) and after the last day of observation (i.e., at the same time that necropsies were performed on fish from test aquaria).

Experiment 3:
Test fish were randomly distributed to each test tank and allowed to acclimate for 2 to 9 days. Test article was dissolved in 50 to 100 mL of tank water and the solution added to the test tank and gently mixed.

Chloramine-T concentration was verified with a Hach DPD colorimeter in triplicate water samples removed from each test tank between 20 to 30 minutes after the test article was added and mixed. Fish were exposed to 180 minute static chloramine-T bath exposures administered once daily for twelve consecutive days. One exposure system consisting of four test tanks (three concentrations and one untreated control; one tank/concentration) was prepared. Temperature, dissolved oxygen, and pH were determined. Total hardness and alkalinity were determined. Flow rates were adjusted daily and recorded.

Mortality was recorded immediately after the initial exposure and before and after each exposure for the following eleven exposures. Following the twelfth exposure, mortality was recorded at 24 hour intervals for 14 days past the twelfth exposure. All remaining fish were euthanized by immersion in ice

water. Wet weight and total length of all fish were determined after death or euthanasia.

Gross necropsy was performed on 12 fish removed from either the acclimation tank or the control tank to serve as initial controls before the first exposure to chloramine-T. Gross necropsies were performed on 12 fish removed from each tank following the last (12th) exposure and on Day 7 and 14 after the last exposure. Hematocrit was determined and blood smears were prepared. Necropsied fish were preserved in 10% neutral buffered formalin and shipped for histopathological screening.

Slides prepared for peripheral blood cytology and gill histopathology were evaluated quantitatively as blood cells and gill lamellae were assessed for lesions or other indicators based on the individual unit. Lesions were classified by the general nature of change (degenerative, hemodynamic, inflammatory, growth, infectious agent), and a more precise qualitative description of the lesion was recorded. Lesions were also evaluated with regard to their extent (focal, multifocal, or diffuse), and their severity was assessed on a scale of 1 to 5 (1 representing minimal change and 5 representing severe change).

3. Statistical Analysis: For each species, life stage, temperature, and exposure time, a generalized linear model was used to test for a group effect of the test article on final cumulative mortality. The ratios dead/total were analyzed as such using a non-linear mixed model with significance level α=0.10. When appropriate, comparison tests of treatment and control mortality rates were done at α=0.10.

4. Results:

   a. Chloramine-T concentration verification and water quality variables:

      Results for the measured chloramine-T concentration showed that the drug administration was appropriate to achieve the target concentrations of 20, 50, 60, 80, 100, and 200 mg/L. Mean dissolved oxygen in treatment aquaria was greater than 5.0 mg/L during all exposures except for isolated instances with walleye fingerlings exposed on four consecutive days.

   b. Cumulative mortality:

      Increasing the exposure concentration, duration, and temperature increased the rate of mortality of channel catfish fry. Increasing the exposure temperature or duration increased the risk of walleye fry mortality at 200 mg/L. A dosage of 20 mg/L for 60 minutes administered on four consecutive days did not produce gross pathological lesions or significantly reduce feeding activity of fry or fingerling freshwater-reared cool- or warmwater fish. Results for cumulative mortality for Experiments 1 and 2 were reported by lifestage, temperature, and exposure duration (Table 20). Results for cumulative mortality for Experiment 3 was reported by lifestage, temperature, and exposure duration (Table 21).

Table 20. Water temperature, age at acclimation, exposure duration of control fish, and cumulative percent mortality at test termination for fish exposed to chloramine-T for 60 or 180 minutes once daily on four consecutive days (n=30 for all test groups).

Species / Life stage	Temp (°C)	Age in weeks	Exposure Time in minutes	% mort. at 0 mg/L	% mort. at 20 mg/L	% mort. at 60 mg/L	% mort. at 100 mg/L	% mort. at 200 mg/L
lake sturgeon fry	20	5-7	60	0	0	0	0	0
northern pike fry	20	6-8	60	3.7	0	3.7	3.7	96.7
walleye fry	15	7-8	60	0	0	0	0	0
walleye fry	20	7-8	60	0	0	0	0	0
walleye fry	20	7-8	180	0	0	0	3.3	100
walleye fry	25	7-8	60	0	0	0	0	66.7
walleye fingerling	20	16-17	60	0	0	0	46.7	100
walleye fingerling	20*	10	60	0	0	0	16.7	100
channel catfish fry	22	6-8	60	0	0	0	0	3.3
channel catfish fry	27	6-8	60	0	0	0	0	83.3
channel catfish fry	27	6-8	180	0	0	43.3	100	100
channel catfish fry	32	6-8	60	0	0	0	3.3	100
channel catfish fingerling	27	12	60	0	0	0	3.3	66.7
largemouth bass fry	27	8	60	0	0	0	6.7	0

*Exposure conducted in ASTM reconstituted soft water (n=18; 6 replicates with 3 fish each).

Table 21. Water temperature, age at acclimation of control fish, and cumulative percent mortality at test termination for walleye or channel catfish fingerlings exposed to chloramine-T for 180 minutes once daily for twelve consecutive days (n=30 for all test groups).

Species / Life stage	Temp (°C)	Age in weeks	% mort. at 0 mg/L	% mort. at 20 mg/L	% mort. at 50 mg/L	% mort. at 80 mg/L
walleye	20	14	1.5	0	0	0
channel catfish	27	16	0	0	0	8.3

c. Histological Results (Experiment 3):

1) Chloramine-T treatment administered for 12 treatments of 180 minutes each elicited no major histopathological effects in walleye fingerlings. Walleye fingerlings were shown to be more sensitive to chloramine-T than walleye fry based on acute toxicity in studies conducted and reported earlier.

2) Lesion severity and extent was not influenced by treatment concentration. There was a shift in lesion category from hemodynamic and inflammatory changes to degenerative changes (erythrocyte necrosis, karryorhexis, or pyknosis) in the walleye spleen as exposure concentration increased. Histopathological changes observed in tissue other than the spleen were relatively unremarkable.

3) Histopathological changes observed in channel catfish fingerlings treated at 20 mg/L were unremarkable compared to controls.

4) The major histopathological change observed was an increase in degenerative changes in the spleen following exposure of channel catfish fingerlings to 80 mg/L. Significantly greater erythrocyte swelling and necrosis was observed in fish treated at 80 mg/L relative to the untreated controls. There were more degenerative changes in the spleen of fish treated at 50 mg/L than in controls; however, the difference was not statistically significant.

5. Conclusions:

a. There is an adequate margin of safety above the proposed maximum label treatment regimen of a 60-minute immersion exposure, administered once daily for three consecutive days at up to 20 mg/L for freshwater-reared cool- and warmwater finfish.

b. Although walleye fingerlings were more sensitive than walleye fry to chloramine-T, an adequate margin of safety exists for the proposed maximum label limit of 20 mg/L.

c. Exposure in reconstituted soft water appeared to have little effect on the safety of chloramine-T to walleye.

E. Supplemental Animal Safety Data:

Type of Study: Depletion of para-toluene sulfonamide from the edible fillet tissue of hybrid striped bass *(Morone saxatilis x M. chrysops)* after exposure to chloramine-T. Study number CAP-00-SBH-05 (November 2001 to January 2002)

Name of Investigator: Jeffery R. Meinertz
U.S. Geological Survey
Upper Midwest Environmental Sciences Center
La Crosse, WI

General Design of Study and Results: The primary study objective was to determine the residue depletion of chloramine-T's marker residue, para-toluene sulfonamide, from the edible fillet of hybrid striped bass following treatment with chloramine-T. Ninety-six hybrid striped bass were treated once daily for 60 minutes on four consecutive days at 20 mg/L (measured concentrations ranged from 18 to 21 mg/L). Chloramine-T was administered as a continuous-flow treatment. Chloramine-T concentration was verified analytically for each exposure by a colorimetric method. All fish were held and treated in one fiberglass raceway. Water temperature before, during, and after exposure was maintained at 15 °C. Fish were removed and euthanized at 0, 12, 24, 48, 96, and 168 hours following the last exposure. Average fish weight was 356.9 g (standard deviation = 84 g) and average total length was 28.1 cm (standard deviation = 1.9 cm). Fish were observed at least once daily for mortality before, during, and after treatment. There were no treatment-related mortalities or adverse reactions reported during the course of the study.

Conclusion: This study supports an adequate margin of safety for chloramine-T administered to hybrid striped bass as a 60-minute bath for four consecutive days at a concentration of 20 mg/L.

IV. Human Food Safety

A. Toxicology:

1. Summary of Toxicology Studies

Based on the evaluation of the residues in edible tissues, we have accepted studies using both chloramine-T (sodium *p*-toluenesulfonchloramide) and its metabolite, para-toluenesulfonamide (*p*-TSA), as being appropriate for the human food safety assessment of chloramine-T.

a. Genetic Toxicity Studies

Study Title: L5178Y $TK^{+/-}$ Mouse Lymphoma Forward Mutation Assay with a Confirmatory Assay with p-toluenesulfonamide
Study Director: Maria A. Cifone.
Study Location: Covance Laboratories Inc. (Covance), Vienna, Virginia.
Study Number: Covance Study No.: 21198-0-431 ICH.
Report Date: May 12, 2000.

The objective of this *in vitro* assay was to evaluate the ability of *p*-TSA to induce forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line. The positive controls were methyl methanesulfonate (MMS - 6.5 and 13 µg/mL) without S9 and methylcholanthrene (MCA - 2 and 4 µg/mL) with S9. Appropriate vehicle controls (dimethylsulfoxide: DMSO) cultures were also exposed in the presence and absence of S9 mix. Rat liver S9 homogenate, prepared from Aroclor 1254-induced male Sprague-Dawley rats, was used in the metabolic activation.

The dose range-finding assay was conducted using a 4-hour dosing period (± S9) and with a 24-hour dosing period (-S9). Dose levels used in each case ranged from 9.85 µg/mL to 5000.0 µg/mL. In the assay performed with a 4-hour exposure period (-S9), the percent relative cell density ranged from 0.0 at 5000 µg/mL to 118.8 at 157.0 µg/mL. Doses below 157.0 µg/mL were not evaluated for the cell density because of lack of toxicity associated with test article treatment. In the presence of metabolic activation, the percent relative cell density ranged from 0.0 at 5000 µg/mL to 95.8 at 9.85 µg/mL. In the assay performed with a 24-hour exposure period (-S9), the percent relative cell density ranged from 0.0 at 5000 µg/mL to 113.9 at 9.85 µg/mL.

Assay #1. Four-hour exposure, -S9: A single trial of the assay (-S9) treated for 4-hours was performed. Eleven concentrations of the test article ranging from 125 to 5000 µg/mL were initiated. Due to excessive toxicity, treatments at and above 2500 µg/mL were terminated. The remaining seven treatments induced weak to high cytotoxicity (76.4% to 19.6% relative growths or RTG). The relative suspension growth (RSG) values ranged from 91.2% at 125.0 µg/mL to 25.9% at 2000 µg/mL. The average mutant frequency of the vehicle control was 47 and the average mutant frequency of the test article treated cultures ranged from 58.6 at 125.0 µg/mL to 80.9 at 2000 µg/mL. None of the analyzed treatments induced increases in the mutant frequency that exceeded the minimum criterion of $>94.0 \times 10^{-6}$ (equal to twice the average mutant frequency of the concurrent vehicle controls that is indicative of a positive response).

Assay #2. Confirmatory assay, -S9: A single trial of the assay (-S9) treated for 24 hours was performed. Eleven concentrations of the test article ranging from 31.3 µg/mL to 3000 µg/mL were initiated. Due to excessive toxicity, treatments at and above 1500 µg/mL were terminated. The remaining seven treatments induced weak to high cytotoxicity (70.6% to 11.7% RTG). The RSG values ranged from 82.7% at 31.3 µg/ml to 13.7% at 1000 µg/mL. The average mutant frequency of the vehicle control was 61.2 and the average mutant frequency of the test article treated cultures ranged from 76.0 at 31.3 µg/mL to 78.7 at 1000 µg/mL. None of the analyzed treatments induced increases in the mutant frequency that exceeded the minimum criterion of $>122.4 \times 10^{-6}$ (equal to twice the average mutant frequency of the concurrent vehicle controls that is indicative of a positive response).

Assay #3. Four-hour exposure, +S9: Eleven concentrations of the test article ranging from 125 µg/mL to 5000 µg/mL were initiated. Due to excessive toxicity, treatments at and above 2500 µg/mL were terminated. The remaining seven treatments were cloned for mutant analysis. These treatments induced no cytotoxicity to moderately high cytotoxicity (133.9% to 29.0% RTG). The RSG values ranged from 112.0% at 125.0 µg/mL to 27.2% at 2000 µg/mL. The vehicle control induced an average mutant frequency of 49.9. Treatment at 2000 µg/mL induced a mutant frequency of 104.3×10^{-6}, which is a 2.1-fold increase over the average vehicle control mutant frequency, indicative of a positive response. The assay was repeated to confirm the initial positive response observed at the top dose of 2000 µg/mL.

Assay #4. Four-hour exposure; Confirmatory Assay, +S9: Eleven concentrations of the test article ranging from 125 µg/mL to 4000 µg/mL were initiated. Due to excessive toxicity, treatments at and above 2250 µg/mL were terminated. The remaining seven treatments were cloned for mutant analysis. These treatments induced no cytotoxicity to high cytotoxicity (116.7% to 14.5% RTG). The RSG values ranged from 110.1% at 500.0 µg/mL to 15.2% at 2000 µg/mL. The vehicle control induced an average mutant frequency of 81.97. Treatment at 2000 µg/mL induced a mutant frequency of 255.8×10^{-6}, which is a 3.12-fold increase over the average vehicle control mutant frequency, indicative of a positive response.

The test article induced forward mutations at the TK locus in the mouse lymphoma cells in the presence of S9 at the top dose of 2000 µg *p*-TSA/mL. This response was also confirmed in an independent assay. The mutant frequency observed at the top dose of 2000 µg *p*-TSA/mL was significantly increased over the concurrent controls in the initial (a 2.1-fold increase over the concurrent control) and confirmatory assay (a 3.12-fold increase over the concurrent control). Therefore, there was a reproducible positive response obtained in the presence of a metabolic activation, and this response is considered as indicative of test article's ability to induce forward mutations at the TK locus in L5178Y mouse lymphoma cells in the presence of S9. The dose at which the positive response was observed exhibited an acceptable cytotoxicity. However, the positive response was at the high dose only and this toxic dose is not considered biologically relevant.

Study Title: *In vivo* Mouse Micronucleus Assay with *p*-toluenesulfonamide.
Study Director: Gregory L. Erexson.
Study Location: Covance Laboratories Inc. (Covance), Vienna, Virginia.
Study Number: Covance Study No.: 21198-0-455OECD.
Report Date: May 23, 2000.

A dose range-finding study was performed using 3 male and 3 female Crl:CD-1 (ICR) BR strain mice treated with the test article (*p*-TSA) at a single dose of 160 mg/kg bw. The test article solution in sterile water was administered to the animals by intravenous route. All animals were examined immediately after dosing, about 1 hour after dosing, and at least

daily for the duration of the assay for toxic signs and/or mortality. The results indicated that all treated mice were slightly hypoactive and exhibited ataxia immediately following dosing. Except for the mortality of a male mouse immediately after dosing, and observation of hypoactive female mice at the 1-hour observation time, no other clinical signs were observed at any other time point. The main study used dose levels of 80, 120, and 160 mg/kg bw. Only male mice were used in the micronucleus assay because there were no substantial differences in clinical observations between the sexes in the dose range-finding assay. The male mice (6 per dose group) were dosed with *p*-TSA or vehicle control by the intravenous route and the positive control (cyclophosphamide, 80 mg/kg bw) by oral gavage.

All animals were examined immediately after dosing, about 1 hour after dosing, and at least daily for the duration of the assay for toxic signs and/or mortality. All animals in the vehicle control and positive control groups appeared normal after dosing and remained healthy until the appropriate harvest time-points. Bone marrow samples were collected from 5 treated animals at the appropriate harvest time-point. Bone marrow sampling times were 24 and 48 hours post-treatment with *p*-TSA and the vehicle control, and 24 hours post-treatment with the positive control. Bone marrow cells stained with May-Grunwald/Giemsa on coded slides were scored for micronucleated polychromatic erythrocytes (MPCEs), and the polychromatic erythrocyte (PCE) to normochromatic erythrocytes (NCE) cell ratio. The micronucleus frequency (expressed as percent micronucleated cells) was determined by analyzing the number of micronucleated PCEs from at least 2000 PCEs per animal. The laboratories normal background micronuclei frequency for ICR mice is reported to be ~0.0-0.4%.

The report states that the treated animals were slightly hypoactive and exhibited polypnea immediately post-dosing. These effects were not observed at later observation times. The test article, *p*-TSA, induced clinical signs of toxicity in the treated animals, but was not cytotoxic to the bone marrow (*i.e.*, no statistically significant decrease in the PCE:NCE ratio). No statistically significant increase in micronucleated PCEs was observed at any dose level or harvest time-point. *p*-TSA was evaluated as negative in the mouse bone marrow micronucleus assay under the conditions of this assay.

Study Title: *In Vivo* Mouse Micronucleus Assay with p-Toluenesulfonamide.
Study Director: Gregory L. Erexson.
Study Location: Covance Laboratories Inc. Vienna Virginia.
Study Number: Covance Study Number 23464-0-455OECD.
Report Date: April 25, 2002.

This study evaluated *p*-TSA for *in vivo* clastogenic activity and/or disruption of the mitotic apparatus by quantifying micronuclei in polychromatic erythrocyte (PCE) cells in Crl:CD-1 (ICR) BR mouse bone marrow. In a dose range-finding assay, 30 males and 30 females, approximately 8 weeks old at dosing were used in the assay. Doses of 0 (vehicle control), 250, 500, 1000, 1500, and 2000 mg/kg bw were administered by oral gavage to 5 mice/sex using a dose limit (up/down) approach. All animals were

examined immediately after dosing, 1 hour after dosing, and at least daily for the next 2 days for toxic signs and/or mortality. No clinical signs of toxicity were observed at 250 mg/kg bw. At 500 and 2000 mg/kg bw immediately post dose, all animals were slightly hypoactive and some exhibited a flattened posture. Some females exhibited labored breathing at 1000 mg/kg bw. At 1500 mg/kg bw, 1 hour post-dose, some animals were recumbent and others were hypoactive and ataxic. At 2000 mg/kg bw, 1 hour post-dose, one female was dead and the rest of the females and all males were recumbent. One day after dosing, all males had recovered but 3 females dosed at 1500 mg/kg bw were either recumbent or ataxic.

The test article was tested in the main study at 4 doses ranging from 187.5 to 1500 mg/kg bw. All treated dose groups were sacrificed at 24 hours post-dose to collect bone marrow. Only the 1500 mg/kg bw group was sacrificed at 48 hours post-dose to collect bone marrow. Slides with bone marrow were scored for PCE to NCE (normochromatic erythrocytes) cell ratio and micronuclei (based on 2000 PCEs). One out of 6 females in the 750 mg/kg bw group and 4 of 18 and 1 of 18 males in the 1500 mg/kg bw group died. At 375 mg/kg, hypoactivity and/or squinted eyes were noted in one animal per sex. At 750 mg/kg bw and 1500 mg/kg bw, animals exhibited hypoactivity, flattened posture, ataxia and/or recumbency. At 1500 mg/kg bw, animals also exhibited labored breathing, were cold to touch, and/or were rolling over to the right. A statistically significant decrease in PCE:NCE ratio was observed with females treated with 750 mg/kg bw. Dose-related depressions in PCE ratio were observed for all animals. However, the numbers of micronuclei (MN)/2000 PCEs were not increased in relation to dose in any animals. These results were indicative that the test article was unable to induce significant increases in the micronucleated PCEs relative to the concurrent vehicle controls under the conditions of the assay.

Study Title: *In Vitro* Chromosomal Aberrations Test of 4-Methylbenzenesulfonamide on Cultured Chinese Hamster Cells.
Study Location: Hatano Research Institute, Food and Drug Safety Center, Cell Toxicology Group, Safety Testing Laboratory, Research Administration, 729-5, Ochiai, Hadano, Kanagawa 257-8523, Japan.

The study used Chinese Hamster Lung (CHL) cells cultured in Eagle MEM broth with 10% fetal calf serum. DMSO was used as a solvent for the test article. The positive control was cyclophosphamide in the presence of S9 mix and mitomycin C in the absence of S9 mix. A dose range finding test used 0.016 to 2.0 mg/mL of test article. The cells were treated for 6 hours in the presence of S9 and continuously for 48-hours in the absence of S9 mix. Growth inhibition of CHL cells by the test article was evaluated by measuring the growth in each test group using the densitometer and comparing with controls. The 50% cell growth inhibition was determined to be 1.3 mg/mL in the absence of S9 mix and 1.7 mg/mL in the presence of S9 mix.

The main study was conducted with 1.3 mg/mL as the top dose in the absence of S9 and 1.7 mg/mL as the top dose in the presence of S9 mix. Three doses without S9 (0.33, 0.65, and 1.3 mg/mL) and three doses with

S9 (0.43, 0.85, and 1.7 mg/mL) were tested. The cells were treated with the test article in the presence of S9 for 6 hours and harvested 24 hours post-treatment, and the cells in the absence of S9 were treated continuously for 24 or 48 hours and then harvested. Colcemid was added to the treated and control cells 2 hours prior to harvesting. Six slides per dish were used to prepare chromosome samples and were stained with Giemsa for scoring for abnormalities. Two hundred cells at mitotic metaphase from each group were scored for structural aberrations and 800 mitotic metaphases were scored for polyploidy. Data was analyzed with Fischer's Exact test. Results were considered positive if the frequency of treated cells with chromosomal aberrations was at least 10% more than the control value and negative if the increase was less than 5% above the control value. Increases of aberration frequencies between 5 and 10% were considered weak or questionable positives. The test article induced significantly increased numbers of cells with polyploidy at a dose of 0.85 mg/mL in the presence of S9, and at 0.65 mg/mL dose in the absence of S9 mix (24 hour continuous treatment). However, there were no positive findings in chromatid abnormalities and the maximal detected percent increase in the numbers of cells with structural aberrations was 3% among the treated cultures, with or without S9. These results did not meet the criteria of a positive response and the test article was negative in the chromosome aberration assay using CHL cells in the presence and absence of a metabolic activation system.

Study Title: Evaluation of the Mutagenic Activity of HALAMID Pharma Grade in the *Salmonella typhimurium* Reverse Mutation Assay and the *Escherichia coli* Reverse Mutation Assay (with independent repeat).
Study Director: C.M. Verspeek-Rip, NOTOX B.V.,
Study Location: s-Hertogenbosch, The Netherlands, September 21, 2000.
NOTOX Project 296674.

The study was conducted using the direct plate incorporation method. Four strains of *Salmonella typhimurium* (*i.e.,* TA 1535, TA 1537, TA 98, and TA100) and one strain of *Escherichia coli* (*i.e*., WP2uvrA) were used in the assay. Metabolic activation system (S9 mix) consisted of liver homogenate from Aroclor-1254 induced rat liver (adult male Wistar rats) and the necessary cofactors. The S9 preparation was evaluated for its metabolic activation capacity by using benzo(α)pyrene. The test substance HALAMID Pharma Grade (chloramine-T) was dissolved in distilled water. Strain specific direct acting mutagens were used to check the sensitivity of the bacterial test strains, and the efficacy of the S9 mix was determined during the test by using a mutagen that required metabolic activation. A preliminary toxicity study using the direct plate incorporation method tested eight concentrations: 3, 10, 33, 100, 333, 1000, 3330, and 5000 µg/plate in strains TA100 and WP2 uvrA in the presence and absence of S9 mix. The test article was toxic to the bacterial strains at 333 µg/plate and above doses in the absence of S9, and in the presence of S9, at 1000 µg/plate and above. The mutagenicity test used 3, 10, 33, 100, and 200 µg/plate in absence of S9, and 10, 33, 100, 333, and 666 µg/plate in presence of S9. All tests used three plates per concentration and solvent, and appropriate positive controls in each test.

The test article was found to be toxic to all the indicator bacterial strains at 200 µg/plate in the absence of S9 and at 666 µg/plate in the presence of S9. Because the initial mutagenicity test was negative, the test was repeated with all the bacterial strains in the presence and absence of the metabolic activation system. The second test used: 10, 50, 100, 150, and 200 µg/plate in the absence of S9, and 100, 200, 300, 400, and 500 µg/plate in the presence of S9. No increases in the mutant counts were observed in the second test and the test article was toxic to some of the tester strains at the top dose in the presence and absence of S9 mix. Halamid Pharma Grade was found not mutagenic in the *Salmonella typhimurium* reverse mutation assay and in the *Escherichia coli* reverse mutation assay.

Study Title: Reverse Mutation Test of 4-Methylbenzenesulfonamide on Bacteria.
Study Location: Hatano Research Institute, Food and Drug Safety Center, Cell Toxicology Group, Safety Testing Laboratory, Research Administration, 729-5, Ochiai, Hadano, Kanagawa 257-8523, Japan.

The study was conducted using the direct plate incorporation method. Five strains of *Salmonella typhimurium (i.e.*, TA1535, TA1537, TA98, and TA100) and one strain of *Escherichia coli* (i.e., WP2uvrA). The S9 mix consisted of liver homogenate from combined phenobarbital and 5,6-benzoflavone induced Sprague-Dawley rat liver and the necessary cofactors. The test substance, 4-methylbenzenesulfonamide (a metabolite of chloramine-T) was dissolved in DMSO at a concentration of 50 mg/mL. Strain specific, direct acting mutagens and a mutagen that requires metabolic activation were used in the test. A dose determination test was performed at 50 to 5000 µg/plate. There was no antibacterial activity at any of the doses tested; therefore, 5 doses ranging from 312.5 µg/plate to 5000 µg/plate were used in the main study. The mutagenicity test was conducted twice in the presence and absence of S9 mix using three plates per concentration, and solvent and appropriate positive controls were used in each test. The positive control mutagens induced large increases in the numbers of revertants. It was concluded that 4-methylbenxenesulfonamide was not mutagenic to the bacterial strains used in the assay.

b. Study Title: Teratology Study in Rats with Chloramine-T (IR-84-238)
Study Location: International Research and Development Corp., Mattawan, MI.
Report Date: 1985
Report Number: 401-290

Healthy Charles River COBS CD rats were randomly selected. Mated dams were accepted upon observation of copulatory plug. Rats were orally gavaged daily with test article in corn oil (10 mL/kg) on Days 6 through 15 of gestation with 0, 50, 250, and 500 mg/kg bw/day. Twenty-five animals/dose were used. Rats were 118 days old at time of mating and ranged 255 to 346 g on gestation Day 0. Animals were housed individually in hanging wire-mesh cages except during mating. A statistically significant decrease in maternal body weight gain was reported in the 250 and

500 mg/kg bw/day groups as compared to the control. Significant weight loss was observed for both treatment groups for the gestation Days 6 to 9. Dams were euthanized on Day 20 of gestation by CO_2 inhalation. No teratogenic effects were observed when administered *via* the oral route to mated Charles River COBS CD rats at the dose levels tested (up to 500 mg/kg bw/day). Maternal toxicity was shown by a significant decrease in body weight gain in the 250 and 500 mg/kg bw/day groups as compared to controls. The following fetotoxic effects were found when compared with controls: an increased incidence of unossification of sternebrae #5 and/or #6 in a number of litters in the 500 mg/kg bw/day group; decreased mean fetal body weights for the 250 and 500 mg/kg bw/day groups; and an increase in post-implantation loss was found in the 250 and 500 mg/kg bw/day groups. A no-observed-effect-level (NOEL) of 50 mg/kg bw/day was determined based on maternal and fetal effects.

c. Study Title: Sub-chronic (90-day) Toxicity Study with HALAMID (Chloramine-T) in Albino Rats
Study Directors: A. de Knecht-van Eekelen and M.I. Williams
Study Location: TNO Central Institute for Nutrition and Food Research, Zeist, The Netherlands.
Report Number: R 3474
Report Date: June 10, 1971

Weanling Wistar derived albino rats were assigned to four test groups based on body weight with 10 animals per sex per group. At treatment initiation, the average weight of males was 63.9 g and average weight of females was 57.9 g. Animals were housed five in a cage. Animals were administered HALAMID (chloramine-T) at diet concentrations of 0, 0.01, 0.03, 0.1 or 0.3% with achieved doses of approximately 0, 10, 30, 100, or 300 mg/kg bw/day.

There were no reported mortalities over the course of the study and no animals were removed for morbidity or moribundity. A significant increase in heart weight in the male 0.1% treatment group and a significant decrease in ovary weight in the female 0.03% treatment group was noted. However, there was no clear dose-related response for the heart and ovary weight differences. There was a statistically significant increase compared with the concurrent control in thyroid weights of female rats at the 0.03% and 0.3% (but not 0.1%) dose levels. However, no histopathological changes were observed in the thyroid that would correlate with the increased thyroid weight. Statistically significant increases in kidney weight were observed in male and female rats in the 0.1% and 0.3% dose groups. In addition, an increased treatment-related incidence in calcareous deposits was noted in the kidneys of female rats of the 0.1% and 0.3% groups. The NOEL for this study was 0.01% chloramine-T in the diet, or 10 mg/kg bw/day.

d. Title: 90-day Oral Toxicity Study with *p*-TSA by Dietary Administration in the Wistar Han Rats
Study Director: F. M. van Otterdijk
Study Location: NOTOX B.V., Hambakenwetering 7, The Netherlands
Study Number: NONTOX Project 474042
Report Date: July 12, 2007

The purpose of this study was to assess the toxicity of *p*-TSA administered *via* dietary admixture to rats during a 90-day dosing period. Wistar Han rats (10/sex/dose) were administered *p*-TSA at diet concentrations of 0, 1000, 3000, and 10000 ppm, designated as group 1, 2, 3, and 4, respectively. Individual animal body weights and food consumption per cage were recorded weekly. Observations of clinical signs of individual animals were conducted daily in cage and weekly outside of cage. Functional observations, performed during week 12-13, consisted of hearing tests, papillary reflex, static righting reflex, grip strength test, and motor activity test. At pre-test and week 13, ophthalmological evaluations were applied to animals in Group 1 and 4. Evaluations of clinical pathology (hematology, coagulation, and clinical biochemistry) were carried out for samples collected on the day of necropsy, following overnight fasting. Animals were killed through exsanguination under isoflurane anesthesia. All animals were examined macroscopically for abnormalities, and the organ weights were measured. Microscopic evaluations were applied to all gross lesions and to all tissues collected from all animals in Groups 1 and 4.

There was no unscheduled animal death during the study. Loss of hair (alopecia) was reported for two males in Group 2 (2/10) and one female in Group 4 (1/10). Based on measured food consumption, the *p*-TSA dose levels were calculated to be 70/80, 214/248, 738/795 mg/kg bw/day for the male/female rats in Groups 2, 3, and 4, respectively. Functional observations yielded no significant positive findings. No ophthalmological changes were found in animals of Group 4 at the end of dosing. Body weight and body weight gains were reduced in both males and females in Group 4; the effect became statistically significant from week 2 for males and week 3 for females, and persisted until the end of the study. During the entire study period, the least weight gains in Group 4 compared to the control group were 21% and 11% for males and females, respectively. There were no significant changes in food consumption for either sex among the treatment groups. For the male rats, in Group 4, the average organ weights of heart, liver, and thymus were reduced by 10, 13, and 21%, respectively, compared to the controls. The relative organ weights of males, normalized to body weight, showed increases for brain (15%), kidney (18%), and testes (14%). For the female rats, no significant changes in absolute organ weight were found in any of the treated groups; the relative organ weights, normalized to body weight, were increased for liver (13%) and spleen (17%). Some statistically significant differences were found in the clinical chemistry parameters between the control and treatment groups. Those changes were, however, limited in magnitude and without consistent dose dependence. Macroscopic evaluations yielded sporadic findings in the male and female reproductive systems: reduced size of testes and epididymides (1 in 10 in both Groups 1 and 4), fluid in the uterus (2 to 3 in 10 in each group, including the control), and ovarian cysts (1 in 10 in Group 2). Histopathological evaluations revealed that 2 in 10 male rats in Group 4 exhibited urothelium hyperplasia in the urinary bladder. There were no positive histological findings in the female groups.

p-TSA was well tolerated at and below the dose level of 3000 ppm (approximately equivalent to 214 and 248 mg/bw/day for male and female

rats, respectively). At 10000 ppm, the highest dose of this study, overt toxicities were observed: persistent decreases in body weight gains for both male and female rats starting from 2 to 3 weeks into dosing, changes in organ weights, and urothelium hyperplasia that is found in the male urinary bladder. Other findings, including changes in clinical pathology parameters, were minor in magnitude and were not dose-dependent. Based on the study results, the NOEL of this study was determined to be 3000 ppm or 214 mg *p*-TSA/kg bw/day.

e. Title: 90-Day Oral Toxicity Study with *p*-TSA by Dietary Administration in Male and Female Beagle Dogs
Study Director: F. M. van Otterdijk
Study Location: NOTOX B.V., Hambakenwetering 7, The Netherlands
Study Number: NONTOX Project 485076
Report Date: April 12, 2008

This study was conducted to assess the toxicity potential of *p*-TSA following 90-day dietary administration in male and female dogs. Young adult Beagle dogs (4/sex/group) were given the diets containing *p*-TSA at the nominal levels of 0, 1000, 3500, and 8000 ppm (achieved doses of 30, 133, and 260 mg/kg body weight/day for the males, and 36, 114, and 255 mg/kg body weight/day for the females, respectively), and were designated as Groups 1, 2, 3, and 4, respectively. The doses were selected following a palatability study and a 28-day dog toxicity study.

The following observations were carried out during the study: mortality (twice daily); clinical signs (once daily) and symptoms; individual body weight (weekly); food consumption (recorded daily and reported as daily average each week); ophthalmoscopic examination for all animals and both eyes during pre-test and week 13; clinical laboratory of blood and urine samples, with blood samples taken from jugular vein and urine samples overnight during pre-test and week 13. At the end of the study, all surviving animals were necropsied and subjected to macroscopic examinations following exsanguination under anesthesia. Organ weight data were recorded, and samples of all major tissues and organs were collected. Histopathological examinations were conducted for the major organ tissues collected from all animals in the control and high dose groups (Groups 1 and 4, respectively). Histopathological evaluations were extended to specific organs of animals in Groups 2 and 3 when macroscopic lesions were observed.

There was no unscheduled animal death. Ophthalmological examinations had no significant findings. Average body weight of male dogs in Group 3 and 4, as well as the corresponding body weight gains, were markedly lower than the controls throughout the treatment period. The body weight data indicate that while the group assignment of dogs introduced some imbalance in body weights among the groups, the *p*-TSA treatment at the higher doses significantly reduced body weight gains of the treated animals. A similar trend of body weight changes, although at smaller magnitude, was also seen in the females. Food consumption of males in Group 4 was reduced (approximately 10% in daily intake, averaged over 13 weeks) for

most of the treatment period; overall food consumption for the females was not significantly affected by the treatment.

No treatment effects were found in clinical chemistry evaluations. No dose-related findings were reported in the urine analyses in either sex. Treatment-related macroscopic findings at necropsy were mostly confined to the stomach. Irregular surface of the pylorus were found in two females in Group 2 and two females in Group 4. Reddish foci were observed in the pylorus and/or cardia region in one female in Group 2 and one female in Group 4. For the male dogs, which showed lower body weight in Group 3 (and to a lesser degree, Group 4), the liver weight in all *p*-TSA-treated groups were lower than the control, reaching statistical significance for Group 4. The average weights of the prostate and epididymis in the treated groups were reduced, with males in Group 3 showing the largest reduction. One dog in Group 3, noted with reduced prostate and thymus size, was reported to have "emaciated appearance". No dose-dependent changes of organ weight were shown in the females of the p-TSA-treated groups.

Histopathological evaluations indicated stomach congestion in one female in each of Groups 2 and 4, corresponding to the reddish foci found at gross examination. In one female each in Groups 2 and 4, lymphoid follicles were found in the stomach area where irregular surface was noted at macroscopic evaluation. In addition, vaginal squamous hyperplasia was found in two female dogs in Group 2, but not in Group 1 or Group 4. Histopathology also revealed diffuse hypertrophy in the zona fasciculata of the adrenals in males and females of all groups. The severity of the lesions ranged from grade 1 (minimal) to grade 2 (slight). The distribution pattern of incidence and severity of the adrenocortical effect across the treatment groups suggests that this lesion was dose-dependent, and the average adrenal organ weight tended to increase with the dose of *p*-TSA.

Significant body and organ weight changes were seen in this study. Even though the changes are not strictly dose-proportional, such effects are likely due to the treatment. Those changes were significant at the dose level of 3500 ppm and above. Histopathological evaluations resulted in positive findings of stomach lymphoid follicles and vaginal squamous hyperplasia. Due to the incompleteness of sample inclusion (not all animals in all four groups were examined), the dose-response relationship of those lesions was difficult to ascertain. The finding of hypertrophy in the zona fasciculata of the adrenals in treated animals showed a plausible dose-dependent progression in both frequency and severity. While hypertrophic changes in the adrenal cortex are often linked to stress response, which is not unusual in experimental animals, the dogs treated with the *p*-TSA showed increased responses compared to the control animals, and this dose-dependence suggests that this finding of adrenocortical hypertrophy is of pharmacological/toxicological significance. Based on the increases of both incidence rate and grade of the histological finding in the adrenals that were seen at the lowest dose level (1000 ppm of p-TSA in diet), a NOEL cannot be established for this 90-day toxicity study in dogs; the lowest-observed effect level (LOEL) was 1000 ppm in diet, or approximately 30 mg *p*-TSA/kg bw/day for this study.

f. Title: Two-Generation Reproductive Toxicity Study in Wistar Rats with *p*-TSA
Study Director: M.E.W. Beekhuijzen
Study Location: NOTOX B.V., Hambakenwetering 7, The Netherlands
Study Number: NOTOX Project 474064
Report Date: November 21, 2011

The purpose of this study was to determine the effects of *p*-TSA on reproductive function and development including gonadal function, estrous cycle, mating behavior, conception, gestation, parturition, lactation, and growth and development of the offspring. The study also provided information on the effects of *p*-TSA on neonatal morbidity, mortality, and maturational development.

p-TSA was administered at dietary concentrations of 1000, 3000, and 10000 ppm to Wistar rats (24/sex/group); the doses were equivalent to 52-78, 165-237, and 566-832 mg/kg bw/day for males and 75-161, 232-499, and 733-1631 mg/kg bw/day for females, respectively. Control animals received diet without *p*-TSA. Treatment of male and female P_0 animals received treatment diets for 70 days prior to cohabitation, then through mating/pairing for a maximum of 15 days, and in females during gestation, parturition and lactation periods; the treatment ended at weaning of P_1 offspring and euthanasia of P_0 animals. F_1 animals, culled on post-partum Day 4, to 4 males and 4 females per litter, were given *p*-TSA in diet after weaning for up to 70 days prior to mating, during cohabitation, during gestation and lactation, and ended at weaning of F_2 and euthanasia of F_1 animals. On Day 4 post-partum, each F_2 litter was culled to 4 males and 4 females. All parental (P_0 and P_1) animals and pups selected for histopathology were anesthetized using isoflurane and subsequently exsanguinated.

Body weights, food consumption, reproduction and breeding data (mating performance, fertility, duration of gestation, implantations, post-implantation loss, number of live/dead pups and breeding/postnatal loss) were collected and analyzed statistically. All rats were necropsied, including unscheduled deaths. Histopathological examination was performed on organs/tissues of 10 randomly selected males and females from the control and high dose groups. A standard set of organs/tissues were collected and examined for possible pathological changes.

The study results indicated that both males and females in each of the two parental generations had increased body weight-adjusted food consumption at all dose levels but reduced body weight at the mid and high dose levels. There were fewer number of implantation sites in the F_1 animals of the mid and high dose groups and reduction in the mean number of live pups in P_0 animals. In P_1 animals, pup viability during postnatal Days 0 to 4 was reduced at the high dose, and gestation index was reduced at mid dose. Increase in the incidence of vaginal epithelial mucification was seen in P_0 and P_1 generations. Increased total number of curled tail sperm cells from the testes of P_0 males and cauda epididymal sperms for both parental generations was recorded. In the P_0, P_1, and F_2 animals, increases or

decreases were seen in the relative weight of a number of organs as normalized to the corresponding body weights.

At the dose level of 3000 ppm dose level, in addition to changes in absolute and relative organ weights of parental offspring animals, the positive findings were reported: decreased terminal body weight in all generations; fewer number of implantation sites in P_1 and reduction in the mean number of live pups in P_0; in P_1, viability index and gestation index were reduced.

At the dose level of 1000 ppm, in addition to sporadic changes in some absolute and relative organ weights, reduced food consumption in males and females of both parental generations and increased anogenital distance in F_2 female pups at birth were also found.

Based on the effects observed at 1000 ppm, including changes in food consumption in males and females of both parental generations, increased organ weight to body weight ratio for thyroid gland and prostate in P_1 male rats, increased anogenital distance in F_2 female pups and decreased absolute thymus weight in F_1 and F_2 females, a NOEL cannot be established for this study; the LOEL was 1000 ppm in diet, or 52 mg *p*-TSA/kg bw/day.

g. Title: Prenatal Developmental Toxicity Study with *p*-TSA in Female NZW Rabbits by Dietary Administration
Study Director: M.H.M. van Tuyl
Study Location: NOTOX B.V. Hambakenwetering 7, The Netherlands
Study Number: NOTOX Project 474097
Report Date: December 14, 2011

This study was designed to determine the potential of *p*-TSA to: 1) induce developmental toxicity after maternal exposure during the critical window of organogenesis, 2) to characterize maternal toxicity at the exposure levels tested, and 3) to determine the NOEL for maternal and embryo/fetal toxicity. *p*-TSA was administered by dietary inclusion at 1000, 3000, and 11000 ppm to female albino New Zealand White rabbits (28/group); the doses were approximately equivalent to 41, 113, and 367 mg/kg bw/day, respectively. Control animals received diet without *p*-TSA. Rabbits were exposed to *p*-TSA from 7 days post-coitum until Day 29 post-coitum (one day prior to necropsy).

Females were fertilized by artificial insemination with sperms collected from two donor bucks of the same strain and source, on the day designated as Day 0 post-coitum. Two batches and a total of 114 female rabbits were inseminated, resulting in a total of 84 pregnant rabbits for inclusion in the study. Clinical observations, body weights, and water and food consumptions were documented in regular intervals. All animals surviving to Day 29 post-coitum were subjected to a clinical examination, then euthanized by intravenous injection of pentobarbital prior to necropsy. External, thoracic and abdominal examinations were performed for possible macroscopic abnormalities. All abnormal tissues were collected for microscopic examinations. Study results were analyzed using standard statistical procedures.

No significant clinical symptoms or unscheduled deaths were reported. At both the 11000 and 3000 ppm dose levels, statistically significant reductions in water and food consumption were noted for certain period during pregnancy. In addition, females treated at 11000 ppm showed a significant decreased in body weight gain from Day 10 onwards compared to the control group. A reduction in relative uterus weight was noted at necropsy in rabbits treated at the 11000 ppm dose level. Prenatal developmental exposure to *p*-TSA did not significantly affect litter size, sex ratio, and body weights at any dose. The percentages of total malformations (external and visceral) found in the dose groups were 15, 0, 15, and 40% for the control, low, mid, and high dose groups, respectively. There were no soft tissue developmental variations attributed to the test substance. The mean percentages of vertebral anomalies were 0, 0.9, 1.3, and 2.4% for the control, low, mid, and high dose groups.

Based on reduction of body weight, body weight gain, food and water consumption in the maternal rabbits, and the vertebral anomaly with or without associated rib anomaly, the NOEL for this study was determined to be 3000 ppm in diet, or 113 mg/kg bw/day.

2. Determination of No Observed Effect Level (NOEL) for chronic exposure

The toxicological Acceptable Daily Intake (ADI) of total chloramine-T-related residues was determined from the lowest NOEL in the most appropriate study among the various toxicology studies conducted. Studies considered in establishing the toxicological ADI are summarized in Table 22.

Table 22. No-Observed Effect Levels (NOELs) or Lowest-Observed Effect Levels (LOELs) in toxicology studies for chloramine-T or *p*-TSA.

Study	Study Number	NOEL/LOEL (mg/kg bw/day) (test substance)
Teratology Study in Rats	IR-84-238	50 (NOEL) (Chloramine-T)
Sub-chronic (90-day) toxicity study with HALAMID in albino rats	R-3474	10 (NOEL) (Chloramine-T)
90-Day Oral Toxicity Study with *p*-TSA by Dietary Administration in Male and Female Beagle Dogs	NONTOX 485076	30 (LOEL) (*p*-TSA)
90-day Oral Toxicity Study with *p*-TSA by Dietary Administration in the Wistar Han Rats	NONTOX 474042	214 (NOEL) (*p*-TSA)
Two-Generation Reproductive Toxicity Study in Wistar Rats with *p*-TSA	NONTOX 474064	52, (LOEL) (*p*-TSA)
Prenatal Developmental Toxicity Study with *p*-TSA in Female NZW Rabbits by Dietary Administration	NONTOX 474097	113 (NOEL) (*p*-TSA)

Based on those toxicology studies, the lowest toxicological NOEL was 10 mg/kg bw/day from the 90-day study in rats.

3. Determination of Toxicological ADI

$$\text{Toxicological Acceptable Daily Intake (ADI)} = \frac{\text{Lowest NOEL}}{\text{Safety Factor}}$$

A safety factor of 2000 was applied based on available toxicology data. Because the lowest NOEL is 10 mg/kg bw/day, the toxicological ADI is calculated as follows:

$$\text{ADI} = \frac{10 \text{ mg/kg bw/day}}{2000}$$
$$= 0.005 \text{ mg/kg bw/day or } 5 \text{ µg/kg bw/day}$$

B. Microbial Food Safety

1. Antimicrobial resistance

Microbial food safety information for chloramine-T for the proposed application was evaluated using a *hazard characterization* approach. The proposed uses are 1) for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp., 2) for the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*, and 3) for the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare.*

Upon evaluation of the information submitted by the sponsor, the Agency determined that chloramine-T is not an antimicrobial drug, and does not share mechanisms of action or resistance mechanisms with any known antimicrobial drugs. There is no reported co- and cross-resistance with existing antimicrobial drugs, and is not considered by the Agency to be important in human medicine at this time. The Agency has determined that there is no identifiable antimicrobial resistance hazard for the use of chloramine-T in freshwater-reared salmonids, walleye, or freshwater-reared warmwater finfish under the proposed conditions of use.

2. Assessment of the impact of chloramine-T residues on human intestinal flora

The safety of chloramine-T related residues in the edible tissues of chloramine-T-treated fish has been assessed for potential impacts on human intestinal flora. A stepwise assessment approach was followed to determine whether there are concerns for residue impact on human intestinal flora.

Step 1: Are residues of chloramine-T and (or) its metabolites microbiologically active against representatives of the human colonic flora?

No, the compound is not active against sentinel bacteria groups. This conclusion was made based on submitted information by the firm of minimum inhibitory concentrations (MIC) cited from previous European Medicines Agency (EMA) approvals. The conclusion is further supported by an *in vitro* susceptibility study recently performed by the Office of Research, FDA/CVM. A summary of the study is provided below.

Study title: Minimum inhibitory concentrations (MICs) of chloramine-T and its metabolite, p-TSA, when testing against recent human isolates of E. coli and enterococci.
Study Number: FDA-CVM # 428.05
Study Report date: 5-17-2011
Study Director: Dr. Maureen Davidson
Study Location: Office of Research, Center for Veterinary Medicine, FDA, 8401 Muirkirk Road, Laurel, MD

Study design: *In vitro* susceptibility testing was performed for chloramine-T and its metabolite, *p*-toluenesulfonamide (p-TSA) using methodology recommended by the *Clinical and Laboratory Standards Institute*. Twelve recent human isolates each of *Escherichia coli* and enterococci were tested. Concentrations for each compound tested ranged from 0.008 to 512 µg/ml. This range should cover tissue concentrations at the toxicological acceptable daily intake (ADI) and its derived safe concentration, as well as the tolerance level.

Results and conclusions: For all 12 isolates of *E. coli*, MICs of chloramine-T and p-TSA were all > 512 µg/ml. For the 12 isolates of enterococci, all had MICs > 512 µg/ml for chloramine-T; while 9/12 were > 512 µg/ml, two isolates had their MICs at 512 µg/ml, and one of them had an MIC at 256 µg/ml for p-TSA. The study concluded that human commensal strains of *E. coli* and enterococci used as sentinel bacterial species had MICs to both chloramine-T and p-TSA that were more than 1,000 times higher than the tolerance limits for p-TSA in fish tissues. Based on the results, it is unlikely that residues of either compound would have any effect on colonic colonization by bacterial species or on development of antimicrobial resistance among bacteria residing in the human intestinal tract.

Step 2: Do chloramine-T residues enter the human colon?

Yes, chloramine-T's metabolite, p-TSA, is the marker residue and it is expected to reach human colon when consumed. However, according to a *National Toxicology Program* analysis of p-TSA absorption, distribution, and excretion after oral administration of ^{14}C-labeled compound to rats, the label was rapidly eliminated largely in urine (66-89% of dose), with little in feces (2-8% of dose)[1]. This finding suggests that the concentration of p-TSA entering in the human colon is indeed very low.

[1] CAS Registry Number: 70-55-3 Toxicity effects. National Toxicology Program, U.S. Department of Health and Human Services (http://ntp.niehs.nih.gov/index.cfm?objectid=E882411D-BDB5-82F8-FE87C65098C4854A, as of April, 2014).

Step 3: Do chloramine-T residues entering the human colon remain microbiologically active?

No, any residues of p-TSA consumed by humans from edible fish tissues would have no apparent antibacterial activity, given the data derived from the OR, FDA/CVM study outlined above.

Conclusions: Compared to the concentration required for inhibiting sentinel bacterial groups representing human intestinal flora, concentrations of chloramine-T and *p*-TSA entering the human colon by ingestion of edible tissues from chloramine-T-treated fish should result in no effects on the human intestinal flora of consumers. Therefore, there is no need to determine a microbiological ADI.

C. Assignment of the Final ADI

Because a microbiological ADI was not needed and therefore, was not calculated, we assign the toxicological ADI (5 µg/kg bw/day) as the final ADI for total chloramine-T residues.

D. Safe Concentrations for Total Residues

The calculation of the tissue safe concentrations is based on the *General Principles for Evaluating the Safety of Compounds used in Food-Producing Animals* (FDA/CVM, revised July 2006). The safe concentration of total chloramine-T residues (ppm) in muscle and/or muscle with adhering skin of freshwater-reared finfish is calculated using the following formulation:

$$\text{Safe concentration} = \frac{\text{ADI}(\mu\text{g/kg bw/day}) \times \text{human body weight}}{\text{grams consumed/day}}$$

$$\text{Safe concentration} = \frac{5\ \mu\text{g/kg bw/day} \times 60\ \text{kg}}{300\ \text{g/day}} = 1\ \text{ppm}$$

E. Residue Chemistry

1. Summary of Residue Chemistry Studies

a. Total Residue and Metabolism Study

Accumulation and clearance of chloramine-T residues in rainbow trout after use pattern treatments with [ring UL-^{14}C] chloramine-T (Study No. LNFRC-CLT-90-01)

Study Dates – April 2, 1990 to May 18, 1990

Study Facility – Upper Midwest Environmental Sciences Center, LaCrosse, WI

Study Director – Verdel K. Dawson

Juvenile rainbow trout (36 to 89 grams) and fingerling rainbow trout (1.8 to 4.5 grams) were immersed one time for 60 minutes in 12 °C water containing 20 mg ^{14}C-chloramine-T/L. Whole body and numerous tissues were collected.

Total residues in the samples were measured by biological sample oxidation, liquid scintillation counting, and HPLC. Metabolic profiling was determined by analysis of the samples with gel permeation chromatography and HPLC. Parent chloramine-T was not found. The major metabolite was *p*-TSA (*para*-toluenesulfonamide).

The edible tissues for fish are muscle with adhering skin except in fish such as catfish and yellow perch where the skin is not eaten. The marker residue to total residue ratio typically is reported for the edible tissue. In this study with rainbow trout, skin from juvenile fish was analyzed only for total residues, not *p*-TSA, and muscle/skin from fingerling fish was analyzed only for *p*-TSA, not total residues. Therefore, the marker residue to total residue ratio is presented for muscle from juvenile fish as an edible tissue and for whole body as a worst-case scenario. The mean marker to total ratios are 95% in juvenile muscle, 76% in fingerling whole body, and 49% in juvenile whole body for timepoints 6 to 240 hours.

Table 23. Total residues and *p*-TSA equivalent residues (mean ± standard deviation or if pooled sample, mean only) in the muscle of juvenile rainbow trout exposed to 20 mg ^{14}C-chloramine-T/L for 60 minutes in 12 ºC water.

Withdrawal Period (hours)	Total Residues (ppm)	*p*-TSA (ppm)	% of *p*-TSA/total residue
1	0.36 ± 0.02	0.14	39
3	0.25 ± 0.02	0.13	52
6	0.18± 0.02	0.12	67
12	0.10 ± 0.04	0.09	90
24	0.08 ± 0.01	0.09	113
48	0.03 ± 0.01	0.03	100
72	0.02 ± 0.01	0.02	100
120	0.01 ± 0.00	0.01	100
240	<0.01	.01	---

Table 24. Total residues and *p*-TSA equivalent residues (mean ± standard deviation or if pooled sample, mean only) in the whole body of fingerling and juvenile rainbow trout exposed to 20 mg ^{14}C-chloramine-T/L for 60 minutes in 12 ºC water.

Withdrawal Period (hours)	Fingerling Rainbow Trout	Fingerling Rainbow Trout	Juvenile Rainbow Trout	Juvenile Rainbow Trout
	Total Residues (ppm)	*p*-TSA (ppm)	Total Residues (ppm)	*p*-TSA (ppm)
1	0.63 ± 0.05	0.26	0.53 ± 0.01	0.15 ± 0.01
3	0.52 ± 0.04	0.21	0.39 ± 0.02	0.15 ± 0.01
6	0.46 ± 0.02	0.24	0.28 ± 0.02	0.13 ± 0.01
12	0.32 ± 0.02	0.29	0.25 ± 0.02	0.11 ± 0.01
24	0.21 ± 0.01	0.15	0.20 ± 0.01	0.08 ± 0.01
48	0.11 ± 0.00	0.08	0.11 ± 0.01	0.05 ± 0.01
72	0.07 ± 0.00	0.05	0.08 ± 0.02	0.04 ± 0.01
120	0.03 ± 0.00	0.03	0.03 ± 0.01	0.02 ± 0.01
240	<0.01	<0.01	<0.01	<0.01

b. Comparative Metabolism Study

Ninety percent of the extractable residue in muscle was *p*-TSA after 12 hours withdrawal and no other significant metabolites were found. Therefore, the requirement for a comparative metabolism study was waived.

c. Residue Depletion Study

Tissue residue depletion studies with chloramine-T were conducted in hybrid striped bass, yellow perch, and rainbow trout to provide residue data for calculating a withdrawal period for all freshwater-reared finfish.

Depletion of para-toluenesulfonamide from the edible fillet tissue of hybrid striped bass, yellow perch, and rainbow trout after exposure to chloramine-T (Nos. CAP-00-SBH-05, CAP-01-YEP-04, and CAP-00-RBT-03, respectively)

Study Dates – November 16, 2001 to January 30, 2002

May 22, 2001 to September 24, 2001

September 18, 2000 to January 9, 2001

Test Facility – Upper Midwest Environmental Sciences Center, LaCrosse, WI

Study Director – Jeffery R. Meinertz

Hybrid striped bass (mean body weight of 357 grams), rainbow trout (mean body weight of 457 grams), and yellow perch (mean body weight of 144 grams) were exposed to 20 mg chloramine-T/L for 60 minutes on 4 consecutive days. The water temperatures were 15 °C for hybrid striped bass, 8 °C for rainbow trout, and 15 °C for yellow perch. Muscle with adhering skin was collected from the fish. The marker residue, *p*-TSA, in the muscle/skin samples was measured using the determinative HPLC method. Concentrations of *p*-TSA were below the tolerance of 0.9 ppm at all sampling timepoints.

Table 25. Mean para-toluenesulfonamide (p-TSA) residues in muscle/skin of hybrid striped bass in 15 °C water exposed to 20 mg/L chloramine-T for 60 minutes on 4 consecutive days.

Withdrawal Period (hours)	*p*-TSA (ppm)
0	0.142 ± 0.030
12	0.159 ± 0.024
24	0.129 ± 0.021
48	0.127± 0.021
96	0.121 ± 0.019
168	0.094 ± 0.011

LOQ = 0.033 ppm

Table 26. Mean para-toluenesulfonamide (p-TSA) residues in muscle/skin of rainbow trout in 8 °C water exposed to 20 mg/L chloramine-T for 60 minutes on 4 consecutive days.

Withdrawal Period (hours)	*p*-TSA (ppm)
0	0.097 ± 0.022
1	0.104 ± 0.017
3	0.099 ± 0.016
6	0.106± 0.016
12	0.092 ± 0.014
24	0.086 ± 0.016
48	0.074 ± 0.009

LOQ = 0.031 ppm

Table 27. Mean *para*-toluenesulfonamide (*p*-TSA) residues in muscle of yellow perch in 15 °C water exposed to 20 mg/L chloramine-T for 60 minutes on 4 consecutive days.

Withdrawal Period (hours)	*p*-TSA (ppm)
0	0.150 ± 0.037
3	0.130 ± 0.032
12	0.150 ± 0.029
24	0.120 ± 0.052
48	0.091 ± 0.031
96	0.064 ± 0.024
168	0.035 ± 0.018

LOQ = 0.024 ppm

2. Target Tissue and Marker Residue Assignment

   The target tissues for fish are muscle with adhering skin except in fish such as catfish and yellow perch where the skin is not eaten. The marker residue for chloramine-T is *para*-toluenesulfonamide (*p*-TSA).

3. Tolerance Assignment

   A tolerance of 0.90 ppm for *p*-TSA, the marker residue for chloramine-T, in the muscle/skin of fish is established. Total residues do not exceed the safe concentration of 1.0 ppm. In consideration that the marker residue/total residue ratio for muscle is 95% and that this ratio does not include skin which is the other edible fish tissue, a tolerance of 0.90 ppm for *p*-TSA is appropriate.

4. Withdrawal Period Assignment

   A withdrawal period of zero days is assigned for chloramine-T in freshwater-reared finfish. Tissue residue depletion data have been provided for three freshwater-reared fish species representing a wide range of cold/cool water temperatures (8 to 15 °C). The tissue residue concentrations of *p*-TSA, the marker residue, are much lower than the tolerance so that any depletion rate differences between fish species are inconsequential. The zero day withdrawal period assigned for chloramine-T in hybrid striped bass, rainbow trout, and yellow perch can be applied to all freshwater-reared finfish.

F. Analytical Methods for Residues

1. Determinative Method

The regulatory procedure for the determination of *p*-TSA, the marker residue for chloramine-T, uses HPLC separation with UV detection.

2. Confirmatory Method

The marker residue, *p*-TSA, is confirmed by GC/MS after derivatization with pentafluorobenzyl bromide. Acceptable confirmation was demonstrated by FDA/CVM/Office of Research.

3. Availability of Methods

The methods are available from CVM, FDA, 7500 Standish Place, Rockville, MD.

V. USER SAFETY:

The product labeling contains the following information regarding safety to humans handling, administering, or exposed to HALAMID Aqua:

On LEFT PANEL:

HUMAN WARNING

EMERGENCY FIRST AID:
- In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contact lenses before flushing eyes and hold the eyelids apart during the flushing. Do not rub eyes. Call a physician. Remove and wash contaminated clothing and shoes promptly and thoroughly. Do not attempt to neutralize with chemical agents.
- If inhaled, move to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.
- If swallowed, do not induce vomiting. Give large quantities of water. Never give anything by mouth to an unconscious person. Call a physician.

INHALATION (Breathing):
- Avoid breathing dust; causes irritation to mucous membranes and may cause asthma-like symptoms.

INGESTION (Swallowing):
- Do not swallow. Swallowing may cause irritation or burns of the mouth, throat, esophagus and stomach that may cause nausea and vomiting.

EYE CONTACT:
- Do not get in eyes; considered corrosive to the eyes.

SKIN CONTACT:
- Avoid contact with skin; considered corrosive to the skin and may cause allergic reaction in sensitive individuals.

HUMAN PRECAUTIONS:
- Keep out of reach of children

- Wear suitable personal protective equipment including gloves, protective clothing and footwear, goggles, and respirator. See product Material Safety Data Sheet (MSDS) for additional information.
- Use only with adequate ventilation
- May aggravate pre-existing skin and/or respiratory disease

On CENTER PANEL:

"Keep out of the reach of children"

VI. AGENCY CONCLUSIONS:

The data submitted in support of this NADA satisfy the requirements of section 512 of the Federal Food, Drug, and Cosmetic Act and 21 CFR part 514. The data demonstrate that HALAMID Aqua, when used according to the label, is safe and effective for the control of mortality in freshwater-reared salmonids due to bacterial gill disease associated with *Flavobacterium* spp.; for the control of mortality in walleye due to external columnaris disease associated with *Flavobacterium columnare*; and for the control of mortality in freshwater-reared warmwater finfish due to external columnaris disease associated with *Flavobacterium columnare*. Additionally, data demonstrate that residues in food products derived from species treated with HALAMID Aqua will not represent a public health concern when the product is used according to the label.

A. Marketing Status:

This product can be marketed over-the-counter (OTC) because the approved labeling contains adequate directions for use by laypersons and the conditions of use prescribed on the label are reasonably certain to be followed in practice.

B. Exclusivity:

HALAMID Aqua, as approved in our approval letter, qualifies for SEVEN years of exclusive marketing rights beginning as of the date of our approval letter. This drug qualifies for exclusive marketing rights under section 573(c) of the Federal Food, Drug, and Cosmetic Act (the act) because it is a designated new animal drug under section 573(a) of the act. Except as provided in section 573(c)(2) of the act, we may not approve or conditionally approve another application submitted for such new animal drug with the same intended use as HALAMID Aqua for the three indications. Because this is the first time we are approving this active ingredient in a new animal drug, this drug also qualifies for five years of exclusivity under section 512(c)(2)(F)(i) of the act. The exclusive marketing rights and exclusivity for this drug run concurrently.

C. Patent Information:

For current information on patents, see the Animal Drugs @ FDA database or the Green Book on the FDA CVM internet website.