

**A TARGET ANIMAL SAFETY STUDY TO DETERMINE:**

**The Safety of Strontium Chloride as a Skeletal Marking Agent for Pacific Salmon**

**Study Number: 2015-001**

- To estimate a margin of safety associated with administering strontium chloride to Chinook salmon fry by immersion (mean fish weight, 0.7 grams) at 0x (0 ppm), 1x (3000 ppm), 3x (9000 ppm), or 5x (15000 ppm) the proposed maximum therapeutic dose of 3000 ppm, for 3x the proposed therapeutic treatment duration of 24 hours.

**Study Objective**

**FIGURE 1.** Photos of the test tanks used during the target animal safety study on Chinook salmon fry at VMC Aquatics Facility.



**Study Room**

**Figure 2.** Photos of labeling procedure for sixteen 1-liter bottles with the sixteen corresponding tank numbers and test article concentrations.



**Bottle Labelling**

- No abnormal behavior of fish in any tank was observed for the 7 days prior to exposure to strontium chloride
- Difficult to distinguish when all the feed offered had been consumed
- Control group, 1x group, and 3x group displayed normal behavior throughout the 3-day exposure period as well as the 24 h post-exposure period.

### Study Results

- During the second 24 hours of exposure, fish in three of the four 5x tanks began to display abnormal behavior generally characterized by reduced appetite, lethargic swimming, and loss of equilibrium and orientation in the water column.
- During the third 24 hours of exposure, fish from all four 5x tanks began to show abnormal behavior.
- In summary, two fish died and 14 fish exhibited signs of morbidity in the 5x exposure group and were euthanized.

### Study Results



### Study Results

Table 2. Summary of mortality and morbidity during the study. Numbers of fish that died/were moribund vs. survived per group

| Group | Moribund/Died | Survived | Mortality |
|-------|---------------|----------|-----------|
| 0x    | 0             | 40       | 0%        |
| 1x    | 0             | 40       | 0%        |
| 3x    | 0             | 40       | 0%        |
| 5x    | 16            | 24       | 40%       |

Mortality within the 5x group

| Yrks | Moribund/Died | Survived | Mortality |
|------|---------------|----------|-----------|
| 3    | 1             | 9        | 10%       |
| 11   | 4             | 8        | 40%       |
| 19   | 2             | 8        | 20%       |
| 18   | 9             | 1        | 90%       |

### Study Results

**Table 3. Summary of gill pathology during the study.**  
 Numbers of mild gill lesions observed per group

| Group | Microbial separation | Regenerative hyperplasia | Regenerative hyperplasia | Regenerative hyperplasia |
|-------|----------------------|--------------------------|--------------------------|--------------------------|
| 0a    | 2                    | 0                        | 0                        | 0                        |
| 1a    | 0                    | 4                        | 0                        | 0                        |
| 2a    | 0                    | 3                        | 0                        | 0                        |
| 3a    | 0                    | 3                        | 0                        | 0                        |
| 4a    | 0                    | 3                        | 0                        | 1                        |
| 5a    | 0                    | 3                        | 0                        | 1                        |

\*One fish observed with moderate amount of lesion affected

Numbers of mild gill lesions observed within the 1a group

| Tank | Microbial separation | Regenerative hyperplasia | Regenerative hyperplasia | Regenerative hyperplasia |
|------|----------------------|--------------------------|--------------------------|--------------------------|
| 3    | 5                    | 9                        | 5                        | 0                        |
| 11   | 2                    | 6                        | 1                        | 0                        |
| 13   | 0                    | 5                        | 0                        | 0                        |
| 23*  | 2                    | 8*                       | 0                        | 1                        |

\*One fish observed with moderate amount of lesion affected

**Study Results**

**FIGURE 3. Mean  $\pm$ SD strontium chloride concentrations in water samples collected during the study.**

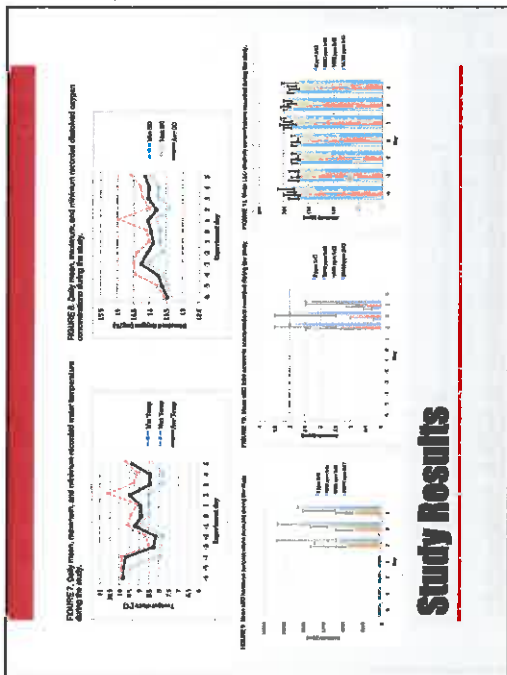
**Study Results**

**FIGURE 4. Percentage difference from target strontium chloride measured in tank water.**

**Study Results**

**FIGURE 5. Mean  $\pm$ SD feet fish weight gain during the study.**  
 No significant difference ( $F=1.3$ ,  $p=0.26$ ) between exposure groups was noted. The single remaining fish from tank #16 was removed as an outlier.

**Study Results**

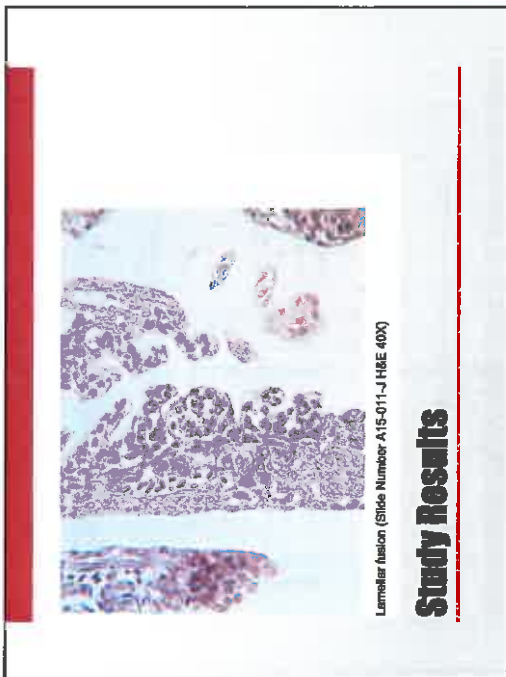
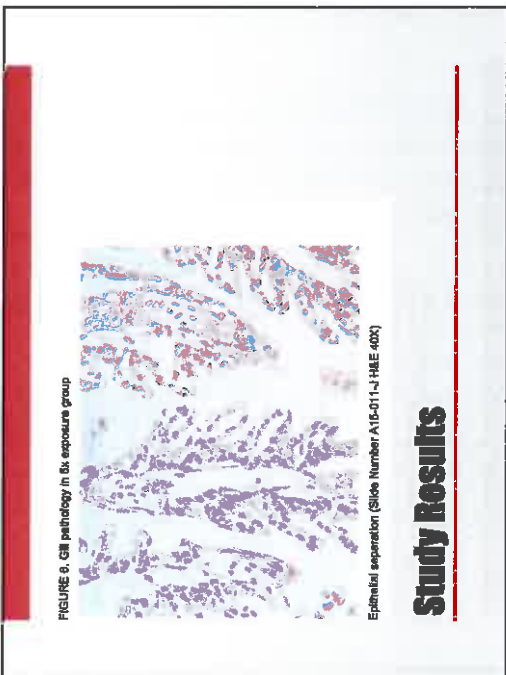


**Study Results**

**Western Chemical, Inc.**  
CERTIFICATE OF ANALYSIS  
Strontium Chloride Hexahydrate

| Lot | Net Weight | Net Content |
|-----|------------|-------------|
| 145 | 1.0000g    | 0.9999g     |
| 146 | 1.0000g    | 0.9999g     |
| 147 | 1.0000g    | 0.9999g     |
| 148 | 1.0000g    | 0.9999g     |
| 149 | 1.0000g    | 0.9999g     |
| 150 | 1.0000g    | 0.9999g     |
| 151 | 1.0000g    | 0.9999g     |
| 152 | 1.0000g    | 0.9999g     |
| 153 | 1.0000g    | 0.9999g     |
| 154 | 1.0000g    | 0.9999g     |
| 155 | 1.0000g    | 0.9999g     |
| 156 | 1.0000g    | 0.9999g     |
| 157 | 1.0000g    | 0.9999g     |
| 158 | 1.0000g    | 0.9999g     |
| 159 | 1.0000g    | 0.9999g     |
| 160 | 1.0000g    | 0.9999g     |
| 161 | 1.0000g    | 0.9999g     |
| 162 | 1.0000g    | 0.9999g     |
| 163 | 1.0000g    | 0.9999g     |
| 164 | 1.0000g    | 0.9999g     |
| 165 | 1.0000g    | 0.9999g     |
| 166 | 1.0000g    | 0.9999g     |
| 167 | 1.0000g    | 0.9999g     |
| 168 | 1.0000g    | 0.9999g     |
| 169 | 1.0000g    | 0.9999g     |
| 170 | 1.0000g    | 0.9999g     |
| 171 | 1.0000g    | 0.9999g     |
| 172 | 1.0000g    | 0.9999g     |
| 173 | 1.0000g    | 0.9999g     |
| 174 | 1.0000g    | 0.9999g     |
| 175 | 1.0000g    | 0.9999g     |
| 176 | 1.0000g    | 0.9999g     |
| 177 | 1.0000g    | 0.9999g     |
| 178 | 1.0000g    | 0.9999g     |
| 179 | 1.0000g    | 0.9999g     |
| 180 | 1.0000g    | 0.9999g     |
| 181 | 1.0000g    | 0.9999g     |
| 182 | 1.0000g    | 0.9999g     |
| 183 | 1.0000g    | 0.9999g     |
| 184 | 1.0000g    | 0.9999g     |
| 185 | 1.0000g    | 0.9999g     |
| 186 | 1.0000g    | 0.9999g     |
| 187 | 1.0000g    | 0.9999g     |
| 188 | 1.0000g    | 0.9999g     |
| 189 | 1.0000g    | 0.9999g     |
| 190 | 1.0000g    | 0.9999g     |
| 191 | 1.0000g    | 0.9999g     |
| 192 | 1.0000g    | 0.9999g     |
| 193 | 1.0000g    | 0.9999g     |
| 194 | 1.0000g    | 0.9999g     |
| 195 | 1.0000g    | 0.9999g     |
| 196 | 1.0000g    | 0.9999g     |
| 197 | 1.0000g    | 0.9999g     |
| 198 | 1.0000g    | 0.9999g     |
| 199 | 1.0000g    | 0.9999g     |
| 200 | 1.0000g    | 0.9999g     |

**Study Results**



**Study Results**

- The null linear mixed model provided sufficient evidence for a higher mortality for fish immersed in the 5x strontium chloride treatment group when compared to the other exposure groups ( $p < 0.0001$ ).
- A dose-related effect on general fish behavior and feeding behavior was observed. Fish in all test tanks consumed 100% of feed, except during days 2 & 3 for the 15000 ppm concentration. Fish in all other test tanks behaved normally.
- No dose-related effect was detected on fish growth ( $F = 1.3, p = 0.26$ ).
- There was a significant difference between the gill lesions found in the 5x exposure group versus the 0x group (Generalized linear mixed model with least squares mean pairwise comparisons,  $p < 0.0001$ ).

## Study Summary

- We conclude that the strontium chloride margin of safety to Chinook salmon fry extends to at least 9000 ppm strontium chloride when administered by immersion for 3 consecutive days.

## Conclusion

Study Number 2015-001 was inspected and monitored by the Quality Assurance Unit of the Animal Health Diagnostic Center at Cornell University to assure conformance with the study protocol, procedures and U.S. FDA GLP regulations.

The inspection dates and dates that findings were reported to the study director and sponsor are listed below.

- 1<sup>st</sup> In - life inspection 20 January 2015
- 2<sup>nd</sup> In - life inspection 26 January 2015
- 3<sup>rd</sup> In - life inspection 27 January 2015
- 4<sup>th</sup> In - life inspection 28 January 2015
- 5<sup>th</sup> In - life inspection 29 January 2015
- 6<sup>th</sup> In - life inspection 30 January 2015
- 7<sup>th</sup> In - life inspection "last" and "After-life" 1<sup>st</sup> and last January 2015

Reviewed and signed raw data records Final Report Audit - 4/3/2015 (Draft)  
QA Unit Review Denise L. Archer

## Quality Assurance



## Study Participants



NRSP-7 North Central Region Report  
May 18, 2015

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**Goat CIDR-G Effectiveness**

Study data has been shipped to the FDA/CVM for comment. Synchronization efficacy looks fine but the reproductive safety data is somewhat mixed.

**Fenbendazole Target Animal Safety in Pheasants**

We have received a technical section complete letter from ONADE. A paper covering this work and the reproductive safety data has been published in Avian Diseases. An update on the efficacy component and an environmental impact statement are needed.

**Lasalocid in Pheasants Target Animal Safety**

We have a technical section incomplete letter from ONADE. We were waiting on the outcome of the fenbendazole TAS technical report before re-submitting this one. Now that the fenbendazole TAS has a technical section complete letter, we should be able to re-submit the lasalocid study report. The work has been published in the Avian Diseases journal.

**Lasalocid in Pheasants Human Food Safety**

The study protocol for the in-life phase at Iowa State was submitted from the Southern Region and we have received protocol concurrence. The FDA had questions on the analytical method and we had planned to complete method validation beginning in June, 2013. The method validation is on-hold pending guidance from the FDA primarily concerning requirements for GLP studies for the MUADP. The study itself has been given a lower priority due to lack of funding and uncertainty over GLP issues.

**Ivermectin Cattle Fever Tick Efficacy**

This project is being done in conjunction with Tom Vickroy in the Southern Region and a whole host of individuals with the Texas Animal Health Commission, the USDA-APHIS and the Cattle Fever Tick Eradication Program as well as with Postive Feeds, Ltd. Two herds (pastures) have been totally cleared of cattle fever ticks and additional data is continuing to be collected on pastures with low tick burdens on which the cattle were fed the ivermectin-containing tubs. A study report covering the data we have to date is being prepared by Dr. Matthew Messenger for submission to the FDA/CVM. The issue of access to the right of reference from Merial is remains unresolved but Merial and Postive Feeds, Ltd met in late April 2015 and apparently agreed to changes in terms of a contract. The Chemistry, Manufacturing and Controls component has not been addressed.

**Fenbendazole in Quail**

*for eyeworms ; quail a big industry in TX*

This is a new project. A product development meeting is planned in the near future to discuss the studies needed for approval.

*eyeworm slows the quail down ;  
hawks get them*

**Draxxin Efficacy in Goats**

This is now largely in the hands of the FDA/CVM and Zoetis.

*- No more work by  
Ron at this time.*

**Pregnant Mare Serum Gonadotrophin-ADR 0353**

A request was received to investigate the feasibility of performing studies to support FDA/CVM approval for Pregnant Mare Serum Gonadotropin to be used as a reproductive aid in small ruminants. A current review of the literature is being prepared with the goal of subsequently requesting a product development conference. No further action at this point.



**NRSP-7 Southern Region Report**  
**Spring 2015 Meeting**  
*May 18, 2015*

**I. PROJECTS**

**A. Lasalocid in Pheasants Target Animal Safety**

We have a technical section incomplete letter from ONADE. We were waiting on the outcome of the fenbendazole TAS technical report before re-submitting this one. Now that the fenbendazole TAS has a technical section complete letter, we should be able to re-submit the lasalocid study report. The work has been published in the Avian Diseases journal.

**B. Lasalocid in Pheasants Human Food Safety**

The study protocol for the in-life phase at Iowa State was submitted from the Southern Region and we have received protocol concurrence. The FDA had questions on the analytical method and we had planned to complete method validation beginning in June, 2013. The method validation is on-hold pending guidance from the FDA primarily concerning requirements for GLP studies for the MUADP. The study itself has been given a lower priority due to lack of funding and uncertainty over GLP issues.

**C. Ivermectin Medicated Feed Block for Control of Cattle Fever Tick in South Texas Quarantine Zone (ADR#352)**

This project is being done in conjunction with Dr. Ron Griffith in the North Central Region of NRSP-7 and a large group of individuals associated with the Texas Animal Health Commission, the USDA-APHIS, the Cattle Fever Tick Eradication Program and a privately held feed company, Postive Feeds, Ltd., headquartered in Texas. Passive exposure of cattle herds has been achieved with use of ivermectin-containing tubs that contain a proprietary but undisclosed mixture of nutrients, minerals, other ingredients and molasses as a taste enhancer. Field studies that have been completed in two separate pastures have led to near complete eradication of cattle fever ticks and additional data is being collected for cattle that are grazing on pastures with low tick burdens. A study report covering the data we have to date is being prepared by Dr. Matthew Messenger for submission to the FDA/CVM. Work conducted thus far at UF has continued with analytical determination of ivermectin content in samples of medicated feed blocks. Analyses have been carried out using a modified version of the approved regulatory method for ivermectin determination in beef liver samples. The method has not yet undergone FDA review for concurrence. The issue of access to the right of reference from Merial is remains unresolved but Merial and Postive Feeds, Ltd met in late April 2015 and apparently agreed to changes in terms of a contract. The Chemistry, Manufacturing and Controls component has not been addressed.

**II. NRSP-7 WEBSITE**

In the time since our most recent annual program meeting in June of 2014, the NRSP-7 website was completely reorganized and updated with a listing of all ongoing studies, completed studies and a streamlined appearance. To date we have received no feedback as to the perceived impact from our target audiences.

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## CVM Project Update Spring 2015 MUADP Meeting

| Project/ INAD   | EFF  | TAS  | HFS  | ENV  | Comments   |
|---|--|--|--|--|--|
| <b>Erythromycin Salmonids</b><br><b>I-006013</b><br><b>CMC incomplete</b> | TS Complete<br>9/28/1998<br>(P-0047)<br><br>TS Complete<br>Reaffirmed<br>4/30/12;<br>Indication for<br><b>Chinook salmon</b><br><b>only (P-0117)</b>                         | TS Complete<br>9/28/1998<br>(P-0047)<br><br>TS Complete<br>Reaffirmed<br>11/7/13;<br>Indication for<br><b>Chinook salmon</b><br><b>only (P-0122)</b> | TS Complete<br>1/11/2007<br>(P-0104)<br><br>(complete for<br>residue and<br>microbial food<br>safety, sponsor to<br>address tox) | EA complete<br>pending revision<br>by CVM (current<br>due date<br>7/20/2015)   | EA hopefully can be done by FDA;<br>After that NRSP-7 will explore<br>extending indication beyond Chinook<br>salmon.<br><br>Requested meeting with Bimeda to<br>discuss the progress of the CMC<br>technical section. Want to make them<br>aware of availability of MUMS grant<br>funding. |
|   | Working on<br>putting together<br>effectiveness<br>study data.<br><br>Synchronization<br>efficacy looks<br>fine but the<br>reproductive<br>safety data is<br>somewhat mixed. | TS Complete<br>2/20/2008   | TS Complete<br>8/12/2011<br>(Milk and tissue<br>residue)   | TS Complete<br>3/19/2012   |  |
| <b>Fenbendazole Pheasants</b><br><b>I-010062</b>                          | Reaffirmation of<br>effectiveness<br>required<br><br>TS initially<br>complete under<br>P-005644-A-0000   | TS Complete<br>11/24/2014  | HFS TS complete<br>on 4/4/2013 -- will<br>need to reference<br>existing<br>toxicology data                                       | Request for Cat<br>Ex not accepted<br>by CVM 7/1/2013<br>-- Working with<br>Mark M. at Merck<br>to have<br>information<br>resubmitted under<br>their INAD. | HFS and Environmental TSs have been<br>affected by activity with this compound<br>in major species.  |
|   | TS complete<br>under PMF 5644<br>in 2000.<br>Will argue that<br>TS should still be<br>considered<br>complete.  | Pending<br>Predevelopment<br>meeting to be<br>scheduled with<br>ONADE  | Pending<br>Predevelopment<br>meeting to be<br>scheduled with<br>ONADE  | Pending<br>Predevelopment<br>meeting to be<br>scheduled with<br>ONADE  | Reviving work on the old claim and<br>introducing a new proposed claim for<br>quail on hunting ranches.<br><br>Working in collaboration with Dr. Ron<br>Kendall of Texas Tech primarily on TAS<br>and HFS technical sections.  |

## CVM Project Update Spring 2015 MUADP Meeting

| Project/ INAD  | EFF   | TAS   | HFS   | ENV   | Comments   |
|--|---|---|---|---|--|
| Lasalocid Pheasants<br>I-009096                                      | TS Complete<br>7/1/2012                             | Study not accepted by CVM 1/30/2013 – need to respond to comments                                   | Protocol Conc 10/31/2012<br>Need to validate method and conduct residue study   | TS Complete<br>2/4/2015<br>P-0026                                     | Method validation work is eligible for MUMS grant funding.<br><br>Many of the TAS incomplete comments are identical to those in the fenbendazole TAS incomplete letter.                                |
| Ivermectin block<br>Cattle<br>I-012056<br><b>CMC incomplete</b>      | Long-term pilot study being conducted by USDA-APHIS | Right of reference pending  | Right of reference pending  | Right of reference pending  | USDA-APHIS is compiling existing effectiveness data for submission to ONADE. Work o other technical sections is on hold pending the right of reference.<br><br>TAS study received OMUMS grant funding. |
| Strontium chloride<br>Salmonids<br>I-010536<br><b>CMC incomplete</b> |   | Protocol concurrence 9/26/2013<br>TAS study completed. Rod currently working on final study report. |   |   |  |
| Tulathromycin<br>Goats<br>I-011512                                   | Design of effectiveness study still undetermined    | TS Complete<br>11/5/2010  | Study not accepted by CVM 4/18/2013 – need to respond to comments<br><br>If HFS is to be done, entire animal study needs to be repeated | TS Complete<br>10/15/2012   | Recommended that Zoetis terminate the designation.   |
| Tulathromycin<br>Sheep<br>I-011513                                   |   |   |   | TS Complete<br>10/15/2012<br>(Request resubmitted under Zoetis' INAD) | Not planning to pursue this one.<br><br>Zoetis terminated the designation.   |

## CVM Project Update Spring 2015 MUADP Meeting

| Project/ INAD                             | EFF | TAS | HFS  | ENV | Comments   |
|---|-----|-----|--|-----|--|
| <b>Nuflor Gold<br/>Sheep<br/>I-011836</b> |     |     | Tissue Residue<br>Study Accepted<br>2/11/2011<br><br>Still need to<br>address microbial<br>food safety |     | Complicating issue of carcinogen<br>potential of the excipient |

