

Inspection Report

SOP No. GEN 001.6

Upper Midwest Environmental Sciences Center

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Quality Assurance Unit

Form GEN 001.6a

Study Director: Maren Tuttle-Lau		Distribution: study director, branch chief, center director
Study Number: AEH-09-MAS-02		Inspection Date(s) June 29, 2012
Location of Inspection: QAO and Maren Tuttle-Lau at UMESC; Sue Schleis and Mike Wellens at Spirit Lake National Fish Hatchery, Spirit Lake, Iowa		Inspection Type: Phone audit; field study
Item	Finding(s) and/or Actions Recommended	Comment(s) and/or Action Taken <sup>1</sup>
	The QAO conducted a phone audit on Friday June 29, 2012 at about 1:30 pm with Maren Tuttle-Lau, Sue Schleis and Mike Wellens. The QAO asked a series of questions based on the study protocol and schedule of events. The questions and answers are shown below.	Note: This study is a clinical trial for Investigation New Animal Drugs, which does not fall under the auspices of Good Laboratory Practice regulations. However, in clinical trials, there is a study monitor to "oversee" the work being conducted. The UMESC QAO was listed as the study monitor and asked to conduct a phone audit in lieu of an on-site visit and is issuing this report to document it.
1.	Q: What study are you conducting? Do you have a copy of the protocol?  A: AEH-09-MAS-02 and title and yes, they have a copy of the protocol, which was locked up in the car with study records.	1. NRN. Study was identified correctly and field staff had a copy of the protocol.
2.	Q: When did you start the experimental treatment?  A: Monday, June 25 about 2:00 pm	2. NRN. Experimental start was consistent with scheduled of events.
3.	Q: How many treatments are you conducting?  A: 10 feedings with Aquaflor, Terramycin or control.	3. NRN. Treatments were as stated in the protocol.
4.	Q: What are the test chemicals?  A: Oxytetracycline and Florfenicol (Terramycin and Aquaflor)	4. NRN. The test articles were correctly identified.
5.	Q: What species of fish are in this trial?	5. NRN. In accordance with protocol that the species would be identified after a natural outbreak of a

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Date Report Issued: June 29, 2012	Study Director Signature:
Response Due Date: July 13, 2012	Management Approval/Date:
<sup>1</sup> NRN = No Response Needed	QAU Review/Date <span style="float: right;">Page 1 of 4</span>

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	A: Walleye	Motile Aeromonad infection.
6.	Q: Who and how were the diseased fish diagnosed?  A: The hatchery notified Maren and she plated samples to get diagnosis confirmed.	6. NRN. Diagnosed according to protocol.
7.	Q: How were the animals randomized?  A: Randomized according to a SAS-generated list.	7. NRN. As described in the protocol.
8.	Q: How are the different feeds kept separate to ensure the proper treatments?  A: Separated by container and labeled	8. NRN. Description appeared to be adequate in preventing cross-contamination or incorrect dosing.
9.	Q: What water quality are you testing and how often?  A: Temperature, dissolved oxygen and pH daily; alkalinity and hardness once during the test period and once post-treatment.  Note: The protocol also mentioned weekly flow rates. Were flow rates weekly?	9.
10.	Q: Explain the experimental units. Are they as detailed in the protocol?  A: The test tank unit was described as in the protocol. 2-Ten tank units, 18 tanks in use (6 each per two treatments of control)	10. NRN. As described in the protocol.
11.	Q: How many days will you dose the fish?  A: 10 days	11. NRN. As described in protocol and schedule of events.
12.	Q: How old are the fish?	12. NRN. Appeared consistent with protocol statement the fish would be juvenile fish equal or

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	A: She thought they were from a spring hatch	less than one year old.
13.	Q: Have there been any mortalities in any of the tanks? If so, which treatments or control?  A: Yes, but could not specify if the mortalities were treatment or control due to the study blinding.	13. NRN.
14.	Q: What personnel are conducting the trial and collecting data?  A: Sue Schleis, Maren Tuttle-Lau, and Mike Wellens	14. NRN. Confirmed by phone.
15.	Q: How are your data safeguarded?  A: Locked in car	15. NRN. Data are being safeguarded.
16.	Q: What is the process for collecting microbiological samples?  A: The process was described.	16. NRN. As described in the protocol
17.	Q: What is the mean weight of the fish you are testing?  A: About 4 grams on Monday, June 25 <sup>th</sup> ; about 2 grams at initial distribution.  Note: The protocol stated the fish were expected to be 5 to 100 grams, but due to the nature of these outbreaks, the weight cannot be planned.	17. NRN.
18.	Q: How many replicates per treatment?  A: 6	18. NRN. As described in the protocol.
19.	Q: How many fish did you use for disease	19.

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	confirmation? A: 34, but protocol said 30 Recommendation: document this as deviation.	
20.	Q: Have you had any problems with equipment, water flow, aeration, etc. that could affect your results?  A: Before the start of dosing, the fish in one tank died when the water flow became clogged and water chemistry changed; the mortalities were removed and the tank refilled; fish were then taken from the remaining tanks to replace the fish that died  Recommendation: Document this as a deviation	20.

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Adverse event recording form

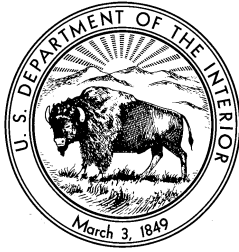
**Adverse Drug Experience:** Record any adverse event associated with the use of a new animal drug, whether or not considered to be drug related.

TANK ID	STUDY DAY	DATE	EVENT DURATION	DESCRIPTION OF ADVERSE EVENT	DRUG RELATED			OBSERVED BY:	STUDY MONITOR NOTIFIED?				
					Yes	No	Unsure		Yes	No	NA <sup>1</sup>	BY:	DATE
B1	9	4 JUL 12	Discovered <sup>were</sup> @ 1315; OK @ 1030	Water flow to tank reduced to drips = 15 mortalities	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	SMS/MPW	<input checked="" type="checkbox"/>	N	NA	MPW	7/4/12
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		
					<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="checkbox"/>	N	NA		

Comments:

Reviewed by Investigator: \_\_\_\_\_ Date: \_\_\_\_\_

<sup>1</sup> Not Applicable



United States Department of the Interior

U.S. GEOLOGICAL SURVEY  
Biological Resources Division  
**Upper Midwest Environmental Sciences Center**  
**2630 Fanta Reed Road**  
**La Crosse, Wisconsin 54603**

## MEMORANDUM

Date: 27Jun12

To: The Record Study Number AEH-09-MAS-02

Subject: Deviation 1 and 2 to the study AEH-09-MAS-02 "Field effectiveness of Aquaflor® (florfenicol) and Terramycin 200 For Fish® (oxytetracycline dihydrate) to control mortality in coolwater and warmwater finfish due to Motile Aeromonad infections."

Deviation #1 – Section 4.5 of the study protocol states that "Fish will be randomly distributed to the test tanks according to a completely random assignment code provided by the Supervisory Biologist. Groups of < 5 fish will be transferred from the appropriate source tank by UMESC study personnel into the assigned test tank according to the random distribution code until each tank has no less than 20 fish and no more than 100 fish per tank depending upon fish loading densities of the source tank. Fish in the source population will be crowded into an area and netted from that crowded population to ensure fish are indiscriminately collected". Approximately 10 fish were netted at a time to provide fish for test tanks.

When the fish were distributed to tanks, it was decided to stock the tanks at a much higher density than the density that the hatchery uses. The amount of fish required in each tank to create a high density situation caused concern for the number of times fish would be netted into and out of the tank. It was then decided to increase the number of fish to 10 instead of 5 per round. I expect no significant impact to the outcome of this study due to the increase from 5 fish to 10 fish per tank or increasing the density from the source tank. We increased the density of the tanks to help create more ideal conditions for the disease to break. Fish were exhibiting some clinical signs and mortality was slightly elevated. Increasing the density of the tanks would accelerate the disease process.

Deviation #2 – Section 4.5 of the study protocol states that "Fish will be randomly distributed to the test tanks according to a completely random assignment code provided by the Supervisory Biologist. Groups of < 5 fish will be transferred from the appropriate source tank by UMESC study personnel into the assigned test tank according to the random distribution code until each tank has no less than 20 fish and no more than 100 fish per tank depending upon fish loading densities of the source tank. Fish in the source population will be crowded into an area and netted from that crowded population to ensure fish are indiscriminately collected".

In anticipation for the study to start after fish were distributed to tanks, the disease did not break until over a week later. Fish were distributed to tanks on 15Jun12 and disease symptoms did not occur again until the week of 23Jun12. On 25Jun12 a second pre-study inspection was complete and fish were redistributed to tanks. To account for the loss of one tank (BX), an appropriate number of fish were removed to leave 50 fish in each tank. The excess were placed in a common vessel to allow for redistribution. The excess were then randomly placed in each tank to have XX in each tank. I do not expect any significant impact of this deviation to the outcome of the study. The study requires an even amount of fish in each tank and redistributing fish allowed for the study to continue.

cc: UMESC QAU

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Maren Tuttle-Lau, M.S.  
Principal Investigator, UMESC

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Date