



DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration  
Rockville MD 20857

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APR 27 2007

U.S. Department of the Interior  
Fish and Wildlife Service  
Aquatic Animal Drug Approval Partnership Program  
Attention: David Erdahl, Ph.D.  
Branch Chief, AADAP  
4050 Bridger Canyon Road  
Bozeman, MT 59715

Re: Isoeugenol (AQUI-S) preliminary target animal safety study on largemouth bass

Dear Dr. Erdahl:

The target animal safety section for the use of isoeugenol to sedate freshwater-reared finfish to handleable remains incomplete. We reviewed your submission dated September 13, 2006, which requested the review of a final study report entitled "Evaluation of the Effects of Life Stage on the Safety of AQUI-S as an Anesthetic to Largemouth Bass *Micropterus salmoides*" and find these data to be acceptable. This study supports the conclusion that small fingerling largemouth bass are more sensitive to overexposure to isoeugenol than are larger fingerling largemouth bass. To complete the technical sections for coolwater and warmwater species of freshwater-reared finfish, you will need acceptable studies in two representative species from each temperature group. To complete the target animal safety technical section for all freshwater-reared salmonids, you will need one additional study in a salmonid species other than rainbow trout. The target animal safety technical section for the use of isoeugenol to sedate all species of freshwater-reared finfish will be complete upon acceptance of studies in two species from each temperature group as agreed upon in the meeting held on February 7, 2006.

ADDITIONAL COMMENTS

1. Based on the data presented in this final study report, as well as that from previously accepted effectiveness studies in your INAD, we infer that earlier life stages (fry-fingerling) of coolwater finfish may be more sensitive to the effects of isoeugenol than later (juvenile) life stages of coolwater finfish. Therefore, we believe that early life stage finfish would be the most appropriate life stage choice for any other species of coolwater finfish used in future target animal safety studies.
2. Based on information in the CVM letters dated February 8, 2005 (P-0067), and February 16, 2007 (P-0128), in which CVM concurred that smaller life stage rainbow trout and warmwater finfish are more sensitive than larger life stage rainbow trout

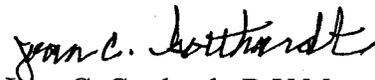
and warmwater finfish when exposed to isoeugenol, as well as the information in this final study report (P-0134), CVM believes that early life stage finfish would be the most appropriate life stage choice for any other species of cold-, cool-, and warmwater finfish used in future target animal safety studies.

#### FOI SUMMARY COMMENTS

A revised FOI Summary section is enclosed. We revised your summary of the study according to CVM's current format for style and content.

If you submit correspondence relating to this letter, you should reference this letter by date and the principal submission identifier(s) found at the top of this letter. If you have any questions about this letter, please contact me at 301-827-7571, or Dr. Donald Prater, Leader, Aquaculture Drugs Team at 301-827-7567.

Sincerely,



Joan C. Gotthardt, D.V.M.  
Director, Division of Therapeutic  
Drugs for Food Animals  
Office of New Animal Drug Evaluation  
Center for Veterinary Medicine

Enclosure: FOI Summary

### III. TARGET ANIMAL SAFETY:

#### A. Toxicity Study

Title: "Evaluation of the Effects of Life Stage on the Safety of AQUI-S as an Anesthetic to Largemouth Bass *Micropterus salmoides*"

Study Director: James D. Bowker, MS

Study Investigators: Daniel Carty and Molly P. Bowman

Study Location: Miles City State Fish Hatchery  
P.O. Box 756  
Miles City, MT

General Study Design:

1. Purpose: To determine the most sensitive life stage of largemouth bass to overexposure to 60 mg/L isoeugenol at a water temperature of approximately 21 °C.
2. Animals: Small fingerling largemouth bass (total length ranged from 5 to 8 cm) and large fingerling largemouth bass (total length ranged from 9.5 to 13.5 cm).
3. Test article: 50% isoeugenol (AQUI-S)
4. Study Design:

**Part 1:** Groups of five (5) small fingerling largemouth bass were exposed to 60 mg/L isoeugenol to establish the longest and shortest exposure times that resulted in 100 and 0% survival. Once these times were determined, exposures were repeated several times to verify that exposure times would consistently result in the desired level of survival.

**Part 2:** Duplicate groups (n = 10/group) of both small fingerling and large fingerling largemouth bass were exposed to 60 mg/L isoeugenol at 6 different exposure times. Using the shortest exposure time of 20.0 minutes and the longest exposure time of 40.0 minutes, a six-point geometric progression equation was used to determine four intermediate exposure durations. Exposures consisted of placing a group of 10 fish into a 60 mg/L isoeugenol solution for one of the six predetermined durations. At the end of the exposure duration, all 10 fish were transferred to a recovery tank filled with fresh water. Fish in the recovery tanks were monitored for survival for 30 minutes. If a fish did not recover within 5 to 15 minutes, it was considered "dead". After fish were designated as "recovered" or "dead", all recovered fish were returned to fish rearing tanks and kept separate from fish that had yet to be used in the study.

5. Measurements and Observations: Mortality was evaluated as the primary variable. Fish behavior during treatment was recorded.

Results:

**Part 1:** The longest exposure time that consistently resulted in 100% survival was 20.0 minutes and the shortest exposure time that consistently resulted in 0% survival was 40.0 minutes.

**Part 2:** The most sensitive life stage was determined by evaluating mortality data and identifying the life stage in which more mortality occurred at a given exposure duration.

Table 1: Mean percent mortality of small fingerling and large fingerling largemouth bass (n = 2 replicate per life stage) exposed to 60 mg/L isoeugenol at a water temperature of approximately 21 °C.

Time in Solution (min)	Mean percent mortality (%)	
	small fingerling	large fingerling
20.00	0	0
22.97	5	0
26.39	20	0
30.31	25	0
34.82	75	0
40.00	95	15

Small fingerling largemouth bass experienced greater mortality than large fingerling largemouth bass across six time points from 20.0 minutes to 40.0 minutes.

Adverse Effects: Other than mortality there were no adverse events reported.

Conclusions: Small fingerling largemouth bass are more sensitive to isoeugenol than large fingerling largemouth following overexposure to 60 mg/L at a water temperature of approximately 21 °C.