



.I-010541-P-0128

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

FEB 16 2007

Re: Isoeugenol (AQUI-S) preliminary target animal safety study on blue catfish

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 July 6, 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 Blue Catfish *Ictalurus furcatus*" and find these data to be acceptable. This study supports the conclusion that fingerling blue catfish are more sensitive to overexposure to isoeugenol than are juvenile blue catfish. 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. We agree with your inference that earlier life stage catfish are more sensitive than later life stage catfish to the effects of isoeugenol.
2. We agree that fingerling channel catfish are an appropriate warmwater life stage to be used for future target animal safety studies performed in support of a New Animal Drug Application (NADA) for isoeugenol.
3. 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 warmwater fish may be more sensitive to the effects of isoeugenol than later (juvenile) life stages of warmwater fish. Therefore, we believe that early

life stage finfish would be the most appropriate life stage choice for any other species of 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 Blue Catfish *Ictalurus furcatus*"

Study Director: James D. Bowker, MS

Study Investigators: Daniel Carty and Molly P. Bowman

Study Location: Fish Breeders of Idaho, Inc.
4647D River Road
Buhl, ID 83316

General Study Design:

1. Purpose: To determine the most sensitive life stage of blue catfish to overexposure to 60 mg/L isoeugenol at a water temperature of approximately 30 °C.
2. Animals: Small fingerling blue catfish (total length ranged from 5 to 6 cm) and juvenile blue catfish (total length ranged from 15 to 20 cm).
3. Test article: 50% isoeugenol (AQUI-S)
4. Study Design:

Part 1: Groups of five (5) small fingerling blue catfish 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 fingerling and juvenile blue catfish were exposed to 60 mg/L isoeugenol at 6 different exposure times. Using the shortest exposure time of 11.5 minutes and the longest exposure time of 23.5 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 11.5 minutes and the shortest exposure time that consistently resulted in 0% survival was 23.5 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 juvenile blue catfish (n = 2 replicate per life stage) exposed to 60 mg/L isoeugenol at a water temperature of approximately 30 °C.

Time in Solution (min)	Mean percent mortality (%)	
	small fingerling	juvenile
11.50	0	0
13.27	20	0
15.30	30	0
17.65	65	5
20.37	85	15
23.50	100	0

Small fingerling blue catfish experienced greater mortality than juvenile blue catfish across six time points from 11.5 minutes to 23.5 minutes.

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

Conclusions: Small fingerling blue catfish are more sensitive to isoeugenol than juvenile blue catfish following overexposure to 60 mg/L at a water temperature of approximately 30 °C.



United States Department of the Interior

FISH AND WILDLIFE SERVICE



AQUATIC ANIMAL DRUG APPROVAL PARTNERSHIP PROGRAM
4050 BRIDGER CANYON ROAD
BOZEMAN, MT 59715
(406) 587-9265/FAX 582-0242

July 6, 2006

Dr. Joan Gotthardt
Director, Division of Therapeutic Drugs
for Food Animals
Document Control Unit, HFV-199
Center for Veterinary Medicine
7500 Standish Place, MPN-2
Rockville, MD 20855

Dear Dr. Gotthardt:

The purpose of this letter is to request a formal review of the enclosed Final Study Report (FSR) titled "Evaluation of the Effects of Life Stage on the Safety of AQUI-S[®] as an Anesthetic to Blue Catfish *Ictalurus furcatus*." The objective of this preliminary study was to determine whether or not there was a life stage effect in blue catfish with respect to their ability to recover from anesthesia following overexposure to 60 mg/L AQUI-S[®]. Please note that we also request that the FSR be included in the AQUI-S[®] target animal safety technical section in support of a New Animal Drug Approval for AQUI-S[®], and that the FSR be filed in the Service's Investigational New Animal Drug (INAD) file 10-541. We refer to your file number INAD 10-541 E-0026 dated January 30, 2003.

The enclosed FSR demonstrates that small fingerling blue catfish (~ 5-6 cm total length) were more sensitive to overexposure to 60 mg/L AQUI-S[®] than larger blue catfish (~ 15-20 cm total length). Although we acknowledge that there may be slight differences relative to efficacy and safety of AQUI-S[®] with respect to different species of catfish, we hypothesize that the relationship relative to life stage sensitivity is consistent between species. Hence, we further hypothesize that, in general, smaller catfish are more sensitive to overexposure with AQUI-S[®] than are larger catfish. Therefore, we contend that results from this FSR support the use of small fingerling channel catfish as an appropriate test fish for future, pivotal AQUI-S[®] target animal safety (TAS) studies. Please note that a draft Freedom of Information (FOI) summary is appended to the FSR.

Dr. Joan Gotthardt - page 2

The current sponsor of INAD #10-541 is Dr. David Erdahl, Branch Chief, U.S. Fish and Wildlife Service, Aquatic Animal Drug Approval Partnership, 4050 Bridger Canyon Road, Bozeman, MT 59715. We would like to thank you in advance for your time and consideration with respect to the above-described request. If you have any questions, please contact Dr. David Erdahl at (406) 994-9904.

Sincerely,

A handwritten signature in black ink, appearing to read "David Erdahl", written in a cursive style.

Dr. David Erdahl
AADAP Program, Branch Chief

enclosure: 3 copies of a Final Study Report