



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

AUG 21 1990

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Addendum to Elanco fish study on Trifluralin

TO: C. Grubbs, PM (74)
Insecticide and Rodenticide Branch
Environmental Fate and Effects Division (H7505C)

FROM: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

In earlier correspondence (MRID 257117), EEB responded to Elanco regarding a vertebral lesion study conducted on fish exposed to trifluralin. Subsequent to that correspondence, Elanco submitted more information on the lesion assessment method (they arrived the same day the EEB review left the agency). A reply had been verbally transmitted to Elanco representatives; the purpose of this letter is to verify that response.

1. EEB concurs with the use of the new X-ray (enlarged X-ray) machine does allow sufficient resolution to routinely detect vertebral anomalies. EEB also agrees that histological examinations of X-rayed fish are necessary to confirm the presence/absence of vertebral lesions. In addition to a lateral view of the vertebral column, EEB recommended that X-rays be taken from a dorso-ventral perspective on each fish to further check for "bent tail" syndrome.
2. EEB concurs with the use of photographic technique for measurement of fish size.

EEB recommends that all fish not used for residue analyses be retained in the event more data will be needed to resolve any issue regarding the potential effect of trifluralin to fish. These fish must not be discarded until the report has been accepted by the Agency and the Agency agrees to discarding fish carcasses.

DowElanco

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January 16, 1990

Document Processing Desk - RS0179
Office of Pesticide Programs - H7508C
U. S. Environmental Protection Agency
401 M. Street, S. W.
Washington, D. C. 20460

Attn: Mr. Bert Baker

Re: **TRIFLURALIN REGISTRATION STANDARD
CONSORTIUM NO. 59011
PROTOCOL FOR AQUATIC ORGANISMS 72-7**

On November 16, 1989, DowElanco submitted a revised protocol to the Agency for the conduct of a 35-day laboratory study with fathead minnows and trifluralin. This study was designed to determine a no-observed-effect concentration for vertebral anomalies. Since that protocol was submitted, DowElanco has determined that certain techniques now available to us would be appropriate to use for this study. In a meeting with Dr. James Ackerman and other EPA personnel on December 14, 1989, our scientists discussed the potential to use these techniques for this 35-day trifluralin study. Dr. Ackerman said he felt the techniques suggested seemed appropriate for the study, but concurred that we should document proposed changes to the protocol in a letter to the Agency. Two items in the submitted protocol would be modified based on the following information.

1. Under item #3 in the November 16 cover letter to the protocol submission, we indicated that the appropriateness of the radiographic procedure used for the field study would be determined before the laboratory study was initiated. The radiographic procedure used in the field study depends on instrumentation that may result in questionable detection capabilities with the small fish to be used in the proposed laboratory study. Late last November, we became aware of an X-ray machine capable of producing enlargements of small fish. In a special pilot experiment in early December with juvenile fathead minnows, the definition and sensitivity of the new machine was determined by a staff veterinary pathologist (D.V.M., Ph.D.). He concluded that radiographs from this machine had sufficient size and clarity to routinely identify trifluralin-induced vertebral lesions. A sample radiograph is enclosed for your examination. Since histological examination was an alternate means to detect lesions with the old less sensitive radiographic technique, the veterinary pathologist recommended that histological examination be performed in only the small number of instances where radiographic analysis may need to be confirmed.

Mr. Bert Baker
U. S. Environmental Protection Agency
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2. Under item #8 in the cover letter to the protocol submission, we indicated that the length and body depth of each fish would be recorded by hand. Since the November 16 submission, we have done a comparison of measurements collected directly by hand and those taken from an 8 x 10 Polaroid photograph of the same juvenile fathead minnows. The measurements were equivalent. Since hand measurements are tedious, time consuming, and have the potential to result in mistakes that cannot be subsequently corrected, photographs of all fish from this laboratory study would be taken to accurately measure their dimensions and to create a permanent record. A sample photograph is enclosed with comparative information on the two measurement techniques.

We propose, therefore, to amend the section (12) in the protocol to the following:

"At test termination, a minimum of 1/3 of the fish will be randomly selected from each replicate and preserved in formalin. All fish remaining in each replicate will be frozen.

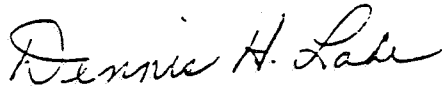
Each fish will be assigned an identification number, weighed, and photographed. Standard length and body depth measurements for each fish will be measured from the photograph.

The fish preserved in formalin will be submitted for radiologic examination. Histological examination will be conducted in those cases where radiographic analysis needs to be confirmed. All frozen fish will be submitted for whole body residue analysis."

We have suggested these techniques and changes to the protocol to improve the quality of the data derived from the proposed study. Upon examination of the information provided, we desire your approval of the proposed amendments to the protocol.

Sincerely,

DOWELANCO



Dennis H. Lade, Ph.D., Project Manager
Plant Science Projects Development and
Registration Division

DHL:lt

Enclosure(s): Radiograph
Photograph and comparison chart

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COMPARISON OF FISH LENGTH AND DEPTH MEASUREMENTS
BY HAND AND FROM A PHOTOGRAPH

Species: Fathead Minnow
Date of Measurement: 11/28/89

Fish #	Standard Length (cm)		Depth (cm)	
	Hand	Photograph	Hand	Photograph
1	3.2	3.3	0.7	0.8
2	2.6	2.7	0.6	0.7
3	2.8	3.0	0.7	0.7
4	2.6	2.7	0.6	0.6
5	2.9	3.0	0.7	0.7
6	2.5	2.6	0.6	0.6
7	2.5	2.6	0.6	0.6
8	2.2	2.2	0.5	0.4
9	2.5	2.6	0.5	0.5
10	2.5	2.6	0.6	0.6
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Mean.	2.63	2.73	0.61	0.62
±S.D.	0.28	0.30	0.07	0.11

