



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

OCT 10 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

OCT 10 1984

SUBJECT: Tackle (Aciflourfen) Audit Report of a Rat Reproduction Study

TO: Richard Mountfort PM-23
Registration Division (TS-767)

FROM: *[Signature]* 10/9/84
Robert P. Zendzian PhD, Acting head
Review Section III
Toxicology Branch
HED (TS-769)

THROUGH: William Burnam, Chief
Toxicology Branch

WAB
10/10/84

Compound Tackle® (acifluorfen)

Tox Chem #818B

Registration #359-TNI

Registrant Rhone-Poulenc

Accession #25415

Action Requested

The Registrant has submitted the following document and requested that the Agency consider it as a basis for upgrading the reproduction study of Tackle® in rats from Invalid to Supplementary.

Three Generation Reproduction Study in Sprague-Dawley Rats Fed Diets Containing Tackle® Herbicide Audit and Final Report of GSRI Project 413-987-41, J.L. Byard and W.L. Spangler Toxicology Consultant Inc & Veterinary Pathology Consultants, Inc. Sept 1984

Conclusion

The extremely low rate of litter production by the F₁ control females and the low rate of litter production by the treated F₁ females coupled with the high incidence of cannibalization of litters indicates a failure of animal husbandry. Further this reproductive failure results in a lack of sufficient control litters to allow evaluation of compound effect on the treated animals. These deficiencies render the study scientifically invalid.

It is also important to note that the high incidence of errors in the initial report strongly suggests that significant error may exist in the raw data. Such error can neither be detected nor corrected.

Background

The Registrant has submitted a 3-generation reproduction study on Tackle® (Gulf South Research Institute, GSRI Project No. 413-987-41, dated November 3, 1982) This study was reviewed by the Agency and classified "Invalid". The following is quoted from the memo reporting the Agency review of this study (Gergorio 3/8/84, copy attached).

"As indicated in the extensive review (attached), reproduction performance of rats being fed TACKLE could not be evaluated based on the data provided (Gulf South Research Institute, GSRI Project No. 413-41, dated November 3, 1982).

Numerous reporting errors, inconsistencies, and omissions were noted throughout the report. In addition, reproductive performance of the control animals was so poor during the first two generations of the study there is no baseline data available for comparisons to the treated animals.

An extremely high incidence of cannibalization among control and treated animals was reported. This behavioral abnormality may reflect a generic problem during the study, such as stress. Stress may be a reflection of poor animal handling and/or husbandry.

These above mentioned problems, more elaborately explained in the attached review, have an adverse impact on the interpretation of this study. Therefore, a definitive assesment of this study could not be made and the Registrant should be apprised that an additional reproduction study is required."

Registrant's Submission

No new data has been submitted. As noted above the Registrant has submitted their own audit report on the study. This report is best described in the words of its authors, "The objective of the audit was to determine the scientific merit of the study, the adequacy of the protocol, and any discrepancies that occurred in the conduct of the study. The GSRI final report was considered by the auditors to be incomplete and to contain a large numbers of errors. Therefore, instead of writing an audit report and correcting the GSRI final report, the auditors have chosen to rewrite the final report, with their audit comments included." (pg 2)

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Discussion

The document submitted is an annotated rewrite of the report of the reproduction study and as such one must first consider the accuracy of this rewrite. If the rewrite is to be used in a detailed analysis of the study, it is necessary that this document be audited by the Agency against the raw data from which it was developed. The history of errors in reporting this study and the possibilities of new errors by writers who had no direct contact with the study but rather worked secondhand from "lab notes" requires this step. However, the original report and the rewrite indicate flaws in the conduct of the study having results which are so fatal to the study as to free us of the necessity of determining how they happened. These fatal results, the failure of controls, will be considered as the primary reason for scientifically invalidating the study and requiring its repetition. Other results indicative of poor performance of the study will also be brought forward.

The experiment under review involves the breeding of three generations of rats and observing the effect of dietary administration of the test compound (Tackle®, acifluorfen) on the process of reproduction. Because of the individual variation inherent in the experimental animals a control breeding group is necessary to demonstrate what can be expected to happen if the experimental animals were not exposed to the test compound. However, in this particular experiment there is an additional reason for a control breeding group. Reproduction is a complex process which is strongly effected by environmental factors. If the animals are not handled properly, not housed properly or not fed properly their reproduction will be adversely effected. Thus, the control group in a reproduction study shows whether the laboratory can breed animals to the extent one would expect under proper conditions of animal husbandry. If the laboratory cannot do this with the control group one can not be certain that the reproductive performance of the treated groups is due to the treatment or to bad animal husbandry. Thus the failure of the control group to reproduce according to expectations in this reproduction study is the fatal flaw which makes the experiment scientifically invalid.

The most important part of a reproduction study is that the females become pregnant and produce litters. The data on maternal reproduction is essentially the same in the two reports and the following is extracted from table 2 of the rewrite.

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Group	Number females Bred	Number Litters	Percent Females Littering
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F₀

Control	26	17	65
25ppm	26	21	81
500ppm	26	20	77
2500ppm	26	26	100

F₁ -> F_{2A}

Control	22	2	9
25ppm	24	7	29
500ppm	24	11	46
2500ppm	27	14	52

F₁ -> F_{2B}

Control	22	10	45
25ppm	24	16	67
500ppm	24	17	71
2500ppm	24	15	62

F -> F_{3A}

Control	21	17	81
25ppm	25	23	92
500ppm	25	20	80
2500ppm	25	22	88

F₂ -> F_{3B}

Control	21	20	95
25ppm	25	23	92
500ppm	24	21	88
2500ppm	25	20	80

The EPA Guidelines on reproduction studies state;

"(B),(iv) Each test and control group should contain at least 20 males and a sufficient number of females to yield at least 20 pregnant females at or near term."

The number of females bred in the F₀ generation was sufficient to produce at least 20 litters in all groups except the controls where the 65 percent littering is considered low. This is already indicative of a husbandry problem.

The situation continued to deteriorate in the breeding of the F₁ control females, with percent littering of 9 and 45 in two consecutive breedings. This is compounded by the higher percent littering in the treated groups of 29(28), 46 and 52 [25, 500 & 2500ppm respectively] in the first breeding and 67, 71 and 62(60) in the second breeding.

Reproduction of the F₂ females can be considered acceptable.

The reduction of littering by the F₀ controls and the almost complete failure of littering by the F₁ controls is indicative of serious problems in animal husbandry. From this data one can conclude that the laboratory was unable to successfully breed rats. The higher percent of littering in the concurrently treated groups can lead to the conclusion that the compound improved reproduction in this study or that the treated animals received better "care" than the controls. The first conclusion is possible but not very likely but the second conclusion would be indicative of a totally unacceptable bias to the study.

The Registrant's sponsored auditors report confirms the conclusion that the laboratory was lacking in experience in rat reproduction. "The GSRI personnel were considered to have adequate training and experience to carry out toxicity studies. However, lack of experience in the areas of rodent reproduction and pathology were evident. The toxicology staff was unable to consistently determine the time of impregnation. The pathology staff drew incorrect conclusions from the pathology incidence data that had not been analyzed statistically. Also, the focus of the GSRI pathologists was on the liver rather than the reproductive organs, which should be of greater concern." (Summary) Further "However, Dr. Barnett stated that the personnel had limited experience in carrying out reproductive studies. The inability of the GSRI personnel to consistently verify mating and their oversight of not confirming pregnancy, indicates lack of expertise in the area of reproductive toxicology." (page 4)

Another indicator of bad husbandry in rat breeding is cannibalization of pups by the dam. The incidence of cannibalization of one or more pups per litter is listed below from the table on page 22 of the rewrite.

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Breeding	Control	25ppm	500ppm	2500ppm
F-0/F-1	2/17	4/21	3/20	6/26
F-1/F-2A	0/2	1/7	2/11	4/14
F-1/F-2B	3/10	2/16	3/17	3/15
F-2B/F-3A	0/17	0/23	0/20	1/22
F-2B/F-3B	2/20	1/23	1/21	1/20

This incidence is considered excessive in rat breeding.

From the information available it is concluded that the conditions for breeding rats in this study were unacceptably poor and the study is scientifically invalid.

Another important consideration must be addressed, both reviews of the original report, EPA's and the Auditor's, agree that the report was rife with errors. The EPA review concluded that the report was unacceptable because of its errors. The auditor decided to rewrite the report because it was uncorrectable. Considering these errors in the report one is forced to assume that the raw data sheets may contain considerable error. Such error can be neither detected nor corrected. On this basis no report based on the raw data sheets can be considered free of error no matter how accurately it presents that data.