IBT Validation Report--Thidiazuron

90-Day Subacute Oral Toxicity Study with SN49537 in Albino Rats

(IBT No. 8560-08337)

Submitted to:

United States Environmental Protection Agency
Office of Pesticide Programs
Hazard Evaluation Division
Toxicology Branch

Thomas Roetzel, Project Officer

Under:

Contract No. 68-01-6561

Dynamac Corporation
Enviro Control Division
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John R. Strange, Project Director

December 31, 1982

IBT VALIDATION REPORT

(1) CHEMICAL: SN 49537 (Thidiazuron).

(2) TYPE OF FORMULATION: Technical, 100% active ingredient.

(3) CITATION: IBT No. 8560-08337. 90-Day Subacute Oral Toxicity Study with SN 49537 in Albino Rats. May 27, 1976.

(4) SPONSOR: Nor-Am Agricultural Products, Inc.

(5) EPA ACCESSION NUMBER and/or Pesticide Petition No. and/or Registration No. for this IBT Report: Registration No. 2139-EUP-23 and Tolerance Petition No. 6G1807.

(6) VALIDATION PERFORMED BY:

John R. Strange, Ph.D.
Department Director
Dynamac Corporation
Signature: John L. Strange
Date: 31 December 1982

Cipriano Cueto, Ph.D.
Program Manager
Dynamac Corporation
Signature: Cipriano Cueto Jr.
Date: 31 December 1982

(7) Based upon findings listed in this Dynamac Corporation validation report (which included examination of the microfiched raw data, the sponsor validation report, the final test report, and the SPRD preliminary report when available), I concur with this validity determination.

Toxicologist
Toxicology Branch
HED, EPA
Signature: Stan O Brin
Date: 1/12/83

Section Head
Toxicology Branch
HED, EPA
Signature: Laurence D. Chitlik
Date: 1/7/83

(8) TOPIC: This study has information pertinent to the discipline of toxicology; topic, subacute feeding. It relates to the Proposed Guidelines data requirement 163.82-1.

(9) VALIDATION REPORT CONCLUSION:

_____ VALID

_X_ SUPPLEMENTARY

_____ INVALID

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SUMMARY

The SPRD report noted that clinical observations, mortality, and gross pathology sheets were not present in the raw data.

The sponsor's validation report noted a few minor errors in food consumption and clinical chemistry data, as well as an error in the concentration of test material in the T-I diet (Deficiency 3b). The sponsor's validator concluded that these had no significant impact on the results of the study.

This validation determines the study to be supplementary because it provides useful information necessary to evaluate subchronic feeding of Thidiazuron to rats. However, the following deficiencies were noted: histopathologic observations were not recorded for all tissues and individual gross pathology/necropsy sheets and clinical observations were not present in the raw data. In addition, diets were prepared only 9 times during the 13-week study instead of weekly intervals and the T-I diet contained only 40 ppm of test material.

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DEFICIENCIES AND DISCREPANCIES NOTED DURING COMPARISONS OF THE FINAL REPORT, PROTOCOL, AND RAW DATA

1. Although the final report stated that representative specimens of 33 tissues and organs were prepared for histopathologic examination (arrow on Reference 1), observations were recorded for only a few (2-4) tissues on the pathology sheets (e.g., Reference 2). It was not possible to determine whether the absence of diagnostic entries for each tissue indicated that the tissue was examined microscopically and found normal or that the tissue was not examined. Consequently, it could not be verified that all the tissues were examined and "no histopathologic alterations attributable to the effects of the test material" were observed as stated in the final report (Reference 3).

2. Although the final report stated that all surviving rats were subjected to a complete gross pathologic examination (arrow on Reference 4), there were no individual gross pathology/necropsy sheets for any of the final sacrifice animals in the raw data. The data for all the animals were recorded in a summary of necropsy findings which are not considered to be raw data sheets (e.g., Reference 5 and Attachment M, pages 1-4).

3. The following deficiencies were noted regarding diet preparation:

   a. Although the final report stated that "fresh diets were prepared each week" (arrow A on Reference 6, page 1), the raw data showed that diets were prepared only 9 times during the 13-week study (arrow B on Reference 7, page 1).

   b. Although the final report indicated that the lowest dietary level used was 60 ppm (arrow on Reference 8), the raw data showed that an error was made during diet preparation. The calculations for the T-I diet showed that 90 g of premix was needed to achieve a level of 60 ppm (arrow F on Reference 9). However, only 60 g of premix (arrow C on Reference 9, and arrow A on Reference 7, page 1) was mixed with 14,940 g rat diet, and consequently the T-I diet contained only 40 ppm of the test material throughout the study.

4. Although the final report stated that "abnormal reactions and/or deaths were recorded daily during the investigation" (arrow B on Reference 6) and "no untoward reactions were noted among any of the animals" (arrow on Reference 10), the raw data did not contain any records indicating that such examinations were conducted. Consequently, it could not be verified that the animals were observed daily for signs of systemic toxicity.

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TOXICOLOGY STUDY PROCEDURES
AS STATED IN THE FINAL REPORT

1. Compound Name and Number: SN 49537, Batch No. 251201B00000.

2. Sponsor: Nor-Am Agricultural Products, Inc.

3. IBT Project No.: 8560-08337.

4. Title of Study: 90-Day Subacute Oral Toxicity Study with SN 49537 in Albino Rats.

5. Laboratory: Industrial-Biotest Laboratories, Inc., Northbrook, IL.

6. Final Report Date: May 27, 1976.

7. Species: Rat.

8. Strain: Albino.

9. No. of Animals: 120.

10. Sex: Male 60 Female 60

11. Source of Animals: Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

12. Age/Weight of Animals at Beginning of Study: The ages of the animals were not given. According to data presented in the results section of the final report, the mean body weights for the control and treatment groups ranged from 136 g to 137 g for the females, and from 150 g to 151 g for the males.

13. Route of Administration (Method of Preparation, etc.): The diet for each group was prepared weekly by blending the appropriate amount of SN 49537 with standard rat ration in a Hobart mixer. Each rat was offered an amount of diet sufficient for 1 week's ad libitum feeding.

14. Experimental Design:

Group	No. of Animals		Dietary Levels (ppm)
	Male	Female	
Control	15	15	None
T-I	15	15	60
T-II	15	15	200
T-III	15	15	600

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15. Clinical Observations Schedule: Abnormal reactions and/or deaths were recorded daily during the investigation.

16. Body Weight Measurement Schedule: Each animal was weighed on the first day of the test and at weekly intervals thereafter.

17. Food Consumption Measurement Schedule: Food consumption was determined weekly and recorded individually for ten rats of each sex in every group.

18. Clinical Studies Schedule: Hematology, blood chemistry, and urinalyses were conducted on 10 rats of each sex from the control and T-III groups after 45 and 84 days of feeding. The parameters measured are indicated by arrow A on Enclosure 1, page 1.

19. Sacrifice Schedule: After 90 days of feeding, all surviving rats were sacrificed.

20. Animals Necropsied (Method of Sacrifice): All surviving rats were rendered unconscious by carbon dioxide, sacrificed by exsanguination, and then necropsied. Animals that died during the study were also examined grossly unless examination was precluded by post-mortem autolysis.

21. Tissues Examined/Preserved at Necropsy: At necropsy various tissues and organs were preserved in 10% neutral buffered formalin. The tissues and organs preserved are indicated by an arrow on Enclosure 1, page 2.

22. Organ Weights Recorded: At necropsy, the weights of the liver, kidneys, spleen, gonads, heart, and brain of each rat were recorded.

23. Animals Whose Tissues Were Examined Microscopically: Tissues taken from 10 rats of each sex from both the control and T-III groups were examined microscopically.

24. Tissues Examined Microscopically: The list of tissues and organs that were examined is indicated by an arrow on Enclosure 1, page 2.

25. Other Procedural Information

    A. Animal Husbandry: Animals were housed individually in standard, wire-bottomed steel rat cages. Each cage bore a color-coded card identifying the animal with respect to project number, dietary level assignment, individual animal number, and sex.

    B. Statistical Analysis: Statistical analyses were conducted on body weight gain and on the absolute organ weights. An analysis of variance was done first, and any significant differences disclosed by that technique were further examined by either

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Tukey's or Scheffe's test of multiple comparison. In addition, organ to body and organ to brain weight ratios were calculated and the data subjected to Kruskal-Wallis' test. "Significant differences disclosed from the treatment were further investigated utilizing Kruskal-Wallis' test of multiple comparison."

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