# IBT Validation Report--Thidiazuron

## 90-Day Subacute Oral Toxicity Study with SN 49537 Technical in Beagle Dogs

(IBT No. 8531-08338)

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: Thidiazuron.

(2) TYPE OF FORMULATION: Technical.

(3) CITATION: IBT No. 8531-08338. 90-Day Subacute Oral Toxicity Study with SN 49537 Technical in Beagle Dogs. June 1, 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 R. Strange
Date: 31 December 1982

Cipriano Cueto, Ph.D.
Program Manager
Dynamac Corporation
Signature: Cipriano Cueto
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: Gary D Burin
Date: 01/31/83

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, subchronic 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 the absence of diet formulation records in the raw data for weeks 11-13 of the study, and a discrepancy in the batch number of the test material used.

The sponsor validation report noted that all the raw data were available to support data presented in the final report, and that a total of 20 minor errors were detected in the final report. The sponsor's validator concluded that none of these errors would change the interpretation of the data and that the findings and conclusions in the final report were accurate and valid.

This validation determines the study to be supplementary because it provides information necessary to evaluate the subchronic oral toxicity of thidiazuron. However, the study is limited because the following deficiencies were noted: it could not be verified that all tissues and organs from each animal were examined histopathologically, or that complete necropsy was performed on all animals. In addition, diet formulation records for weeks 11-13 of the study and clinical observation records for the duration of the study were not present in the raw data.



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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 A on Reference 1, page 1), observations were recorded for only a few (0-9) tissues on the individual pathology sheets (e.g., arrow C on Reference 2). Consequently, it could not be verified that all the tissues were examined and found normal as stated in the final report (arrow on Reference 3).

2. Although the final report stated that a complete gross necropsy was conducted on each dog (arrow B on Reference 1, page 1), no observations were recorded on the individual pathology sheets [redacted] or summary sheet (Reference 4) for 10 of the 32 animals. In addition, only limited information was present on these sheets for the remaining 22 animals. Consequently, it could not be verified that a thorough gross pathologic examination was conducted or that no abnormalities were found as stated in the final report (arrow on Reference 3).

3. Although the final report stated that the diets were prepared each week (arrow A on Reference 5), diet formulation records were available for only the first 10 weeks of the study (e.g., Reference 6 and Attachment A). There were no diet preparation records in the raw data for weeks 11-13 of the study. Consequently, it could not be verified that the diets were prepared for weeks 11-13.

4. Although the final report stated that "the dogs were under observation during the investigation and were examined daily for clinical signs or symptoms indicative of systemic toxicity" (arrow B on Reference 5), and that no unusual behavioral reactions were noted at any dietary level during the investigation (arrow on Reference 7), 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: Thidiazuron Technical, SN 49537.

2. Sponsor: NOR-AM Agricultural Products, Inc.

3. IBT Project No.: 8531-08338.

4. Title of Study: 90-Day Subacute Oral Toxicity Study with SN 49537 Technical in Beagle Dogs.

5. Laboratory: Industrial BIO-TEST Laboratories, Northbrook, Illinois.

6. Final Report Date: June 1, 1976.

7. Species: Dog.

8. Strain: Purebred beagle.

9. No. of Animals: 32.

10. Sex: Male 16 Female 16 .

11. Source of Animals: IBT colony.

12. Age/Weight of Animals at Beginning of Study: The dogs were approximately 5.5 months old. The mean body weights of males and females were approximately 6.1 and 7.7, respectively.

13. Route of Administration (Method of Preparation, etc.): The test material was administered in the diet. The diet was prepared weekly by blending the proper dietary constituents in a Hobart mixer. Food was offered for 5 hours/day, 7 days/week, on an ad libitum basis.

14. Experimental Design:

| Group | No. of Animals | | Dietary Levels (ppm) |
|---|---|---|---|
| | Male | Female | |
| UC | 4 | 4 | 0 |
| T-I | 4 | 4 | 100 |
| T-II | 4 | 4 | 300 |
| T-III | 4 | 4 | 1,000 |



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15. Clinical Observations Schedule: The animals were examined daily for clinical signs or symptoms indicative of systemic toxicity.

16. Body Weight Measurement Schedule: The dogs were weighed at the beginning of the study and then weekly for the duration of the test.

17. Food Consumption Measurement Schedule: Food consumption was determined weekly for each group of animals.

18. Clinical Studies Schedule: The clinical parameters measured (hematology, clinical chemistry, and urinalyses) are listed in Enclosure 1. These parameters were measured just prior to the inception of the study and after 42 and 85 days of testing.

19. Sacrifice Schedule: All animals were sacrificed at the conclusion of the study.

20. Animals Necropsied (Method of Sacrifice): All of the dogs were necropsied. The animals were anesthetized and then sacrificed by exsanguination.

21. Tissues Examined/Preserved at Necropsy: All major tissues and organs were examined grossly. Representative specimens from tissues and organs listed in Enclosure 2 were preserved at necropsy.

22. Organ Weights Recorded: The weights of the following organs were determined: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland, and pituitary gland.

23. Animals Whose Tissues Were Examined Microscopically: A histopathologic examination was performed on representative specimens from the tissues and organs listed in Enclosure 2 for all animals.

24. Tissues Examined Microscopically: The tissues examined microscopically are listed in Enclosure 2.

25. Other Procedural Information

    A. Test Material (Identity, etc.): The test material was identified as SN 49537 Technical.

    B. Animal Husbandry: The beagle dogs were all elible for AKC registration and had been previously immunized against rabies, distemper, infectious canine hepatitis, and leptospirosis. Four dogs of the same sex and dose group were accommodated in a single kennel equipped with outside runs.



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## RESULTS OF RAW DATA REVIEW

I. Administrative Information

A. Protocol (Design and/or Addenda): A protocol dated December 16, 1975 (Reference 8), and prepared by IBT was present in the raw data (arrows on Reference 9). The protocol was in good agreement with the final report procedures. However, the protocol did not specify the doses to be used in the study (arrow on Reference 8).

B. Rationale for Dose Administration Levels: The rationale was based on a pilot study conducted with dietary levels of 0, 300, 1,000, 3,000, or (5,000) 10,000 ppm of test material for 2 weeks (arrows on Reference 10, pages 1 and 2).

C. Letter of Authorization: A letter of authorization dated January 7, 1976, was present in the raw data (arrows on Reference 11). It was sent by Drs. Sellke and Kaiser of Schering AG, West Germany, to M. L. Keplinger of IBT.

II. Compound

A. Identification (Chemical Name, Chemical Number, Lot Numbers): The test material was identified in a shipping receipt as "SN 49537 (Tech. Batch 250601E00000)" (arrow A on Reference 12). However, diet formulation sheets had a different batch number, 251201B0000 (arrow A on Reference 6, page 1).

B. Shipping Receipts: A shipping receipt dated January 6, 1976 (arrow B on Reference 12) and marked received by IBT on January 8, 1976 (arrow C on Reference 12), was present in the raw data.

C. Stability/Special Handling: No information was present in the raw data.

D. Storage: No information was present in the raw data.

E. Analysis: No information was present in the raw data.

F. Purity: Diet formulation sheets indicated that the technical material was 100% pure (arrow B on Reference 6, page 1).



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III. Compound Preparation/Administration

A. Methods of Compound Preparation: The test material was mixed with Purina Dog Chow at a concentration of 1,000 ppm to prepare the T-III diet. This diet was then mixed with the appropriate amount of chow to prepare the T-I and T-II diets (arrow on Reference 6, page 2).

B. Frequency of Compound Preparation: The diet formulation records indicated that diets were prepared weekly for the first 10 weeks of the study (e.g., Reference 6 and Attachment A). There were no diet preparation records in the raw data for weeks 11-13 of the study.

C. Calculations for Compound Preparation: Calculations for diet preparations were present in the diet formulation records (e.g., Reference 6, page 2). These calculations were checked and found to be correct.

D. Storage of Prepared Compound: No information was present in the raw data.

E. Analysis of Prepared Compound: The raw data indicated that no samples were taken for analysis (arrow D on Reference 6, page 1).

F. Administration: Diet preparation records (e.g., Reference 6) and food consumption data (Reference 13) present in the raw data were indirect indications that the test material was mixed in the diet and offered ad libitum.

G. Start of Compound Administration: A diet preparation form indicated that the study was to start on February 12, 1976 (arrow on Reference 14).

H. Termination of Compound Administration: Diet preparation forms indicated that the test diets were last prepared on April 19, 1976; i.e., week 10 of the study (Attachment A). However, food consumption data were present for the 13-week duration of the study (arrow on Reference 13, page 2).

IV. Animal Information

A. Date of Order: No information was present in the raw data.

B. Date of Receipt: An IBT dog shipment data sheet was dated January 29, 1976 (arrow A on Reference 15).

C. Total Number of Animals Ordered:

1. Males: 16.

2. Females: 16.



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D. Species/Strain: The food consumption data sheet indicated that beagle dogs were used in the study (arrow A on Reference 13, page 1).

E. Source: The dogs were obtained from the IBT colony (arrow B on Reference 15).

F. Age/Weight: Whelping dates recorded on the shipment data sheet indicated that the dogs were 5-6 months old at the initiation of the study (arrow C on Reference 15).

G. Duration of Quarantine: No information was present in the raw data. However, the dogs were previously vaccinated against distemper, hepatitis, leptospirosis, and rabies (arrow D on Reference 15) and were shipped 2 weeks before the study started (arrow A on Reference 15).

H. Start of Animal Phase: Day 0 hematology values indicated that baseline values were determined on February 10, 1976 (arrow on Reference 16, page 1), 2 days before the dogs were placed on the test diets (arrow on Reference 14).

I. Termination of Animal Phase: The dogs were sacrificed on May 14, 1976, as indicated in a summary gross pathology sheet (e.g., arrow on Reference 4).

V. Environmental Conditions

There was no information on environmental conditions present in the raw data.

VI. Biological Parameters

A. Body Weights: Body weights were recorded weekly for all animals for the 13-week duration of the study (Reference 17). Individual body weights recorded in the raw data for male and female control and T-III animals were compared with data presented in the final report (Attachment B), and no discrepancies were found.

B. Food Consumption: Food consumption was determined weekly on a group basis for all test groups for the duration of the study (Reference 13, pages 1 and 2). However, the dates on which food consumption was determined were not recorded, except for the first 4 weeks (arrow B on Reference 13, page 1). Values for weekly food consumption recorded for male and female control and T-III animals in the raw data were compared with values presented in the final report (Attachment C). Two incorrect entries were found in the final report.

C. Clinical Observation/Mortality: The raw data contained no clinical or behavioral observations. No mortalities occurred during the course of the study as indicated by the sacrifice date recorded on the individual pathology sheets (e.g., arrow D on Reference 2).

D. Clinical Studies: Clinical studies were present in the raw data for hematology, clinical chemistry, and urinalysis. These studies were conducted on 2/10/76 (e.g., arrow on Reference 16, page 1), 3/29/76 (e.g., arrow on Reference 18), and 5/10/76 (arrow on Reference 19), and corresponded with days -2, 46, and 88 of the study and not with days 0, 42, and 85, as stated in the final report (e.g., arrow on Attachment D, page 1).

1. Hematology: Seven hematologic parameters (WBC, RBC, Hgb, Hct, MCV, MCH, and MCHC) and five differential leukocyte counts (neutrophils, lymphocytes, monocytes, eosinophils, and basophils) were recorded for each animal on days -2, 46, and 88 of the study (e.g., arrows on References 16 and 20). Hematologic parameters were recorded on computer printouts (e.g., Reference 16), whereas differential leukocyte counts were recorded on data sheets (e.g., Reference 20). Values recorded in the raw data for the hematologic parameters and differential leukocyte counts for male and female control and T-III animals on days -2 and 88 of the study were compared wih the values given in the final report (Attachment D), and no errors were found.

2. Clinical Chemistry: Five parameters (glucose, BUN, SAP, SGPT, and SGOT) were recorded for each animal on test days -2, 46, and 88 (e.g., Reference 19). Values for all five parameters reported in the raw data for male and female control and T-III animals on days -2 and 88 of the study were compared with values presented in the final report (Attachment E), and no discrepancies were found.

3. Urinalysis: Raw data were present for seven urinalysis parameters (albumin, glucose, pH, specific gravity, WBC, RBC, and crystals) for each animal on test days -2, 46, and 88 (e.g., Reference 18). Values present in the raw data for test days -2 and 88 for male and female control and T-III animals were compared with values recorded in the final report (Attachment F), and seven discrepancies were found. The raw data indicated that a sample was not taken for control dog No. 4 on day -2 (arrow on Reference 21).

E. Organ Weights: The weights of nine organs (adrenals, brain, gonads, heart, kidneys, liver, pituitary, spleen, and thyroid) were recorded on individual pathology sheets for all 32 dogs (e.g., arrow A on Reference 2). The weights of the nine organs from each animal in the control and T-III groups were compared with those presented in the final report and one discrepancy



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was found (Attachment G). The ratios of organ weights to final body weights and brain weights of all animals were calculated from the raw data and compared with the values in the final report (Attachment G). The computed values were slightly lower (in all cases by a fraction of one unit) than those presented in the final report (Attachment G). The correct values were inserted and rounded to the second decimal point.

F. Gross Pathology/Necropsy: Gross pathology data were recorded on individual pathology forms (e.g., arrow B on Reference 2) and on a summary form (Reference 4). However, observations were not recorded for 10 of the 32 animals (Attachment H). The observations recorded in the raw data for male and female control and T-III animals were compared with those presented in the final report (Attachment H), and no discrepancies were found.

G. Histopathology: Histology data were recorded on individual pathology sheets which were present for all 32 animals (e.g., arrow C on Reference 2). However, observations were recorded for only a few tissues (0-9) from individual animals (e.g., Reference 2), and not the 33 tissues listed in the final report (Reference 1, page 2).

The observations recorded in the raw data for male and female animals in the control and T-III groups were compared with observations presented in the final report (Attachment H), and no discrepancies were found.