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

007950

MAY 31 1990

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Review additional data submitted in support of a chronic toxicity study in dogs, a chronic in rats, and an In Vitro Cytogenetic Assay in Human Lymphocytes with Thidiazuron.
EPA ID No. 45639-89, 456039-89, EPA Record No. 235572, 235572, EPA MRID #. 407983-00 through 407983-04 and 409085-01, 411917-01, HED Project No. 9-0444, 9-2215, Caswell No. 659A.

TO: Miller/DJ and Richard Mountfort, PM # 23
Herbicide-Fungicide Branch
Registration Division (H7505C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 5/28/90
Pharmacologist, Review Section I
Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support/HED (H7509C)

THRU: Yiannakis M. Ioannou, Ph.D., D.A.B.T. J.M. Ioannou 5/23/90
Section Head, Review Section I
and
Marcia van Gemert, Ph.D. Marcia van Gemert 5/23/90
Chief, Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support
Health Effects Division (H7509C)

Registrant: NOR-AM Chemical Company
3509 Silverside Road
P.O. Box 7495
Wilmington, DE 19803

Action Requested: Review additional data submitted in support of a chronic toxicity study in dogs, a chronic in rats, and an In Vitro Cytogenetic Assay in Human Lymphocytes with Thidiazuron; also in support of Conditional Registration of DROPP 50WP 45639-89.

Recommendations: The additional data submitted does not support the Conditional Registration for DROPP 50WP 45639-89. The chronic feeding study in rats is classified as Core-Supplementary Data for oncogenicity and is downgraded to Core-Supplementary for chronic toxicity. The chronic feeding study in dogs remains classified as Core-Supplementary Data pending submission of

additional data requested in this memo. For the in vitro human lymphocyte cytogenetic study, the nonactivated portion of the assay remains unacceptable and the S9-activated assay remains acceptable. **The Toxicology Branch - Herbicide, Fungicide, Antimicrobial Support recommends against the Conditional Registration for DROPP 50WP 34639-89.**

DISCUSSION

Upon review of the additional data submitted in support of a chronic toxicity study in dogs with Thidiazuron, the Toxicology Branch-Herbicide, Fungicide, Antimicrobial Support finds that the data in this submission addresses the requested histology data and individual animal data for ophthalmic findings asked for in the review by Dynamac (DER dated 9/27/89). The present submission also addresses the Agency comment that it may be necessary to do further testing to clarify the possible immunotoxicity effects suggested by the results of this study. The registrant has reassessed the relevant toxicity data and undertook an additional study in dogs to clarify the etiology of the observed hemolytic anemia, however, the data from the additional study were not supplied to the Agency for consideration. These data must be supplied to allow for a complete review of the effects in the dog.

At present, the additional histopathology data support a tentative LOEL of 300 ppm of thidiazuron and a NOEL of 100 ppm of thidiazuron based on hemolytic anemia. An increased incidence of moderate or marked hemosiderosis and an increase in Kupffer cell proliferation was observed in livers of mid and high dose males and in high dose females; marked or moderate hemosiderosis in the spleen and hyperplasia of the erythroid cells in the bone marrow were also observed in the same groups. These findings correlate with the anemia in mid dose males and high dose females.

> **The classification for the chronic toxicity study in dogs remains as Core-Supplementary until the additional data is received by the Agency and reviewed.**

The classification for the chronic toxicity study in rats with thidiazuron is downgraded to Core-Supplementary Data since the effect on body weight gains at the highest dose tested was of equivocal toxicological importance. Therefore, a clear effect level was not established. **The classification for oncogenicity remains Core-Supplementary Data** since the information submitted does not support the contention that a Maximum Tolerated Dose (MTD) was approached. In addition, the rationale for dose selection was not adequate.

Comments were received from the registrant and the testing laboratory regarding the TB-HFAS review of an in vitro human lymphocyte cytogenetic study with Thidiazuron. The DER stated that **the nonactivated portion of the assay was considered to be unacceptable** because the sensitivity of the system for detecting a direct-acting clastogen was questionable, and rare complex aberrations were seen at two assayed doses of the test material. However, **the S9-activated assay was acceptable.** These original conclusions were accurate and therefore, the comments from the registrant do not change our position regarding the unacceptability of the nonactivated test results. The responses to the individual comments are attached.

Previously Submitted Data on Thidiazuron

The following data were submitted prior to this application.

Guideline #	Study Type	Tox.Cat.	Core Classification
Acute Toxicity			
81-1	Acute oral toxicity in rats with technical material there is an unclassified study with the EP available	III	acceptable
81-2	Acute dermal toxicity in rabbits with technical material there is a unclassified study with the EP available	II	acceptable
81-3	Acute inhalation toxicity in rats with technical material there is an unclassified study with the EP available	III	**IBT not validated**
81-4	Primary eye irritation in rabbits with technical material there is an unclassified study with the EP available		**Unacceptable DATA GAP**
81-5	Primary dermal irritation in rabbits with technical material there is an unclassified study with the EP available	IV	acceptable
81-6	Dermal sensitization - guinea pig with the technical material also there are no studies with the MP or EP available		**DATA GAP**

Subchronic Testing	
82-1 90 day feeding study - rat (2 studies)	Minimum
82-1 90 day feeding study - mouse	met by onco
82-1 6 month feeding - dog (2 studies)	Supplementary **DATA GAP**
82-2 21 day dermal - rabbit	**DATA GAP**
Chronic Testing	
83-1 2-year feeding - rodent (2 studies)	Supplementary **DATA GAP**
83-1 1 year feeding - nonrodent	Supplementary **DATA GAP**
83-2 Oncogenicity - rat (2 studies)	Supplementary **DATA GAP**
83-2 Oncogenicity - mouse	Minimum
83-3 Teratology - rat	Minimum
83-3 Teratology - rabbits (2 studies)	Minimum
83-4 Multigeneration reproduction-rat	Minimum
Mutagenicity Testing	
84-2 Dominant Lethal Test in mice	Acceptable
84-2 Ames	Acceptable
84-2 Micronucleus (mouse)	Acceptable
84-2 In Vitro cytogenetics	Acceptable for activated Unacceptable for nonactivated
Special Testing	
85-1 General metabolism - rat	Supplementary **DATA GAP**

The above data gaps must be resolved before additional tolerances can be considered. The database requires extensive reevaluation.

EPA: 68D80056
DYNAMAC No.: 233-C
TASK No.: 2-33C
February 22, 1990

007955

SPECIAL REVIEW

THIDIAZURON

Additional Information Submitted By Registrant
Chronic Study in Rats

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: Feb 22, 1990

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature:
Date: 2/22/90

Stephen C. Dapson, Ph.D.
Pharmacologist, Review
Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Stephen C. Dapson
Date: 2/28/90

Yiannakis M. Ioannou, Ph.D.
D.A.B.T.
Section Head, Review
Section I
Toxicology Branch - HFAS
(H-7509C)

Signature:
Date: 3/2/90

I. SUBMISSIONS:

A. Tennekes, H.A. Thidiazuron: Comments on the review of the 104-week chronic toxicity study with Thidiazuron technical in the rat by the U.S. Environmental Protection Agency (Unpublished report: RCC Project 011924, Research and Consulting Co. AG. Itingen, Switzerland, dated Nov. 18, 1987). MRID No. 407983-03.

B. Sachsse, K. T49 Thidiazuron Addendum: 104-week chronic toxicity and oncogenicity study with thidiazuron technical in the rat. Ophthalmoscopy - Individual Findings (Unpublished Co. AG Itingen Switzerland, dated December 14, 1987) MRID No. 407983-02.

II. CONCLUSIONS:

The classification for chronic toxicity is downgraded to CORE supplementary since the effect on body weight gains at the highest dose tested was of equivocal toxicologic importance. Therefore, a clear effect level was not established. The classification for oncogenicity remains CORE supplementary, since the information submitted do not support the contention that a Maximum Tolerated Dose was approached. In addition, the rationale for dose selection was not adequate.

III. SUMMARY:

Comments:

A. Although the decrements in cumulative body weight gains noted for the group 4 males (600 ppm) were less than 10%, the likelihood is that the highest dose tested in the present study was greater than one-half of the required Maximum Tolerated Dose (MTD). The LOEL for systemic toxicity (600 ppm) in male rats was in fact based on observed changes in body weight.

B. "Survival rate may be affected as doses of greater than 600 ppm and if this change is related to treatment (which could be the case) a meaningful assessment of oncogenicity may be compromised..."

Response to Comments:

A. The MTD is normally established from the results of subchronic toxicity studies. The basis for dose selection for this chronic study was an invalid chronic toxicity study by IBT. No results on subchronic studies (a dose greater than 600 ppm should have been tested) were presented to support the dose selection.

A decreased weight gain did not occur in high-dose males in the first 13 weeks of the study; during this period of maximum growth, weight gains were indistinguishable in dosed and control groups of both sexes. Between weeks 0 to 51 and 0 to 104 the weight gains in the high-dose males were only about 4% lower than in controls. The decreased weight gain in high-dose males is of doubtful toxicologic importance. There is no basis for assuming that the highest dose was greater than one-half of the MTD.

B. Survival at 107 weeks in high-dose males was 85% when compared to 82% for controls. In females, percent survival at all doses was similar (68 to 72%) and Kaplan-Meir calculations indicated 90% survival at 547 days (78 weeks) in females receiving 600 ppm. The days on test for 25% mortality was 682 (97 weeks) for females receiving 600 ppm which was only slightly shorter (735 days or 105 weeks) than the 25% mortality rate in control females.

Since there were no effects of dosing on survival and no trends, it is unlikely that a somewhat higher dose would have resulted in unacceptable mortality. EPA guidelines accept up to 50% mortality at 18 months in chronic studies in rats. In this study the mortality was less than 10% in both sexes at 18 months.

OPHTHALMOLOGIC FINDINGS:

Data for ophthalmologic examinations for 10 rats/sex/group at ~~group~~ at pretest, at 3, 6, and 12 months and at termination were provided. The only finding noted was eye crust in one female receiving 300 ppm at 3 months. There was no similar finding for the same animal at any other interval. It is concluded that there were no effects on dosing on the eyes of any animals.

EPA No.: 68D80056
DYNAMAC No.: 233A-4
TASK No.: 233-A4
May 3, 1990

007950

DATA EVALUATION RECORD

THIDIAZURON

Additional Information Submitted by Registrant
Chronic Toxicity in Dogs

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: May 3, 1990

APPROVED BY:

Robert J. Weir, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Roman J Puentas Jr
Date: may 3, 1990

Stephen C. Dapson, Ph.D.
Pharmacologist, Review
Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Stephen C. Dapson
Date: 5/11/90

Yiannakis M. Ioannou, Ph.D.
D.A.B.T.
Section Head, Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Y M Ioannou
Date: 5-15-90

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007950

SUBMISSION:

Hollihn, U. T48-Supplement 1, Thidiazuron: Systemic tolerance study in dogs following daily administration via the feed over a period of one year. (An unpublished study No. PF 55/84 submitted by Schering AG, Berlin, Federal Republic of Germany, dated June 9, 1989). MRID No. 411917-01.

CONCLUSIONS:

The registrant submitted the EPA requested histology data and individual animal data for ophthalmic findings (MRID No. 407933) reviewed in Dynamac Task No. 2-33A dated September 27, 1989. The present submission is in response to the EPA comment that it may be necessary to do further work to clarify possible immunotoxicity effects suggested in the study. The sponsor has reassessed the relevant toxicology data and undertaken an additional study in dogs to clarify the etiology of the observed hemolytic anemia.

Although reassessment of the original study did not suggest to the sponsor an immunotoxic etiology of the observed anemia, the new data submitted by the sponsor did not address the EPA comment, nor were any data submitted relating to immunotoxicity.

SUMMARY:

Five male beagle dogs were treated by gelatin capsule with 50 mg/kg/day thidiazuron for one week. The dose was progressively increased to 100 and 200 mg/kg/day on succeeding weeks. No methemoglobinemia or affects on other red cell parameters were observed. The dose was then progressively doubled every two days. At 1600 mg/kg, three dogs exhibited reduced erythrocyte counts, hemoglobin levels, and hematocrit values. Methemoglobin levels were marginally increased in two dogs but this was not considered biologically important. Other parameters were not examined for the dogs. In the original study, methemoglobin levels were not measured.

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CONFIDENTIAL BUSINESS INFORMATION
DOES [illegible]
NATIONAL SECURITY [illegible] EO 12065)

EPA No.: 6880056
DYNAMAC No.: 233-A
TASK No.: 2-33A
May 3, 1990

007950

SPECIAL REVIEW

THIDIAZURON

Additional Information Submitted by Registrant
Chronic Toxicity in Dogs

REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: May 3, 1990

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Roman J Prenta for
Date: may 3, 1990

Stephen C. Dapson, Ph.D.
Pharmacologist, Review
Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Stephen C Dapson
Date: 5/11/90

Yiannakis M. Ioannou, Ph.D.
D.A.B.T.
Section Head, Review
Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Y.M. Ioannou
Date: 5/15/90

SUBMISSION:

Khater, A.R. T48 Thidiazuron Addendum: SN49537 systemic tolerance study in dogs following daily administration via the feed over a period of one year: Microscopic examination of all collected organs of group 2 and 3 and the bone (with bone marrow) and the eye of groups 1 and 4. (An unpublished study, No. TX 83.003, performed and submitted by Schering AG, Berlin, Federal Republic of Germany, dated Feb. 2, 1988) MRID No. 407983-01.

CONCLUSIONS:

The registrant submitted requested histology data for ophthalmic findings. The additional histopathology data tentatively support a LOEL of 300 ppm thidiazuron and a NOEL of 100 ppm based on hemolytic anemia. An increased incidence of moderate or marked hemosiderosis and an increase in Kupffer cell proliferation was observed in the livers of mid- and high-dose males and in high-dose females; marked or moderate hemo- siderosis in the spleen and hyperplasia of the erythroid cells in the bone marrow were also observed in the same groups. These findings correlate with the anemia in mid-dose males and high-dose females. Interstitial pneumonia was observed in all groups, was generally mild, and is not considered related to dosing; it does not affect interpretation of study results. However, additional data has been requested relative to the additional study undertaken to clarify the etiology of the observed hemolytic anemia.

SUMMARY:

Table 1 summarizes the histologic findings in all groups of dogs in the study. The only histologic findings that were considered related to dosing were in the liver, spleen, bone marrow, and kidneys. These findings are a compensatory reaction to the hemolytic anemia seen in mid- and high-dose males and females. An increase in moderate or marked hemosiderosis in the liver of mid- and high-dose males and of high-dose females; was observed. A mild increase in Kupffer cells of the liver was also seen in the same groups. Hemo- siderin deposits in the kidney tubules was increased in mid- and high-dose males and in high-dose females. Although some degree of hemosiderosis was seen in the spleens of all dogs, including controls, the severity of the finding was increased in the mid- and high-dose groups. An increase in hyperplasia of the hematopoietic cells of the bone marrow was also observed in mid- and high-dose dogs of both sexes.

EPA No.: 68D80056
DYNAMAC No.: 233-B1
TASK No.: 2-33B1
February 22, 1990

007950

DATA EVALUATION RECORD

THIDIAZURON

Mutagenicity--Response to comments on the review of the mutagenicity evaluation of thidiazuron, Batch No. 7/9.82, in an in vitro cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures (T45 Thidiazuron, Accession No. 262816) by the U.S. Environmental Protection Agency.

APPROVED BY:

Robert J. Weir, Ph.D.
Program Manager
Dynamac Corporation

Signature: Robert J. Weir

Date: 2/22/90

EPA No.: 68D80056
DYNAMAC No.: 233-81
TASK No.: 2-3381
February 22, 1990

007950

DATA EVALUATION RECORD

THIDIAZURON

Mutagenicity--Response to comments on the review of the mutagenicity evaluation of thidiazuron, Batch No. 7/9.82, in an in vitro cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures (T45 Thidiazuron, Accession No. 262816) by the U.S. Environmental Protection Agency.

REVIEWED BY:

Nancy E. McCarroll, B.S.
Principal Reviewer
Dynamac Corporation

Signature: William L. McCarroll for
Date: Feb. 22, 1990

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: for I.C. Felkner
Date: 2.22.90

APPROVED BY:

Roman J. Pienta, Ph.D.
Department Manager
Dynamac Corporation

Signature: Roman J Pienta
Date: 2-22-90

Stephen C. Dapson, Ph.D.
Pharmacologist
Review Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Stephen C. Dapson
Date: 2/28/90

Yiannakis M. Ioannou, Ph.D., D.A.B.T.
Section Head
Review Section I
Toxicology Branch - HFAS
(H7509C)

Signature: Y M Ioannou
Date: 3/2/90

DATA EVALJATION RECORD

037950

CHEMICAL: Thidiazuron.

STUDY TYPE: Mutagenicity--Response to comments on the review of the mutagenicity evaluation of thidiazuron, Batch No. 7/9.82, in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures (T45 Thidiazuron, Accession No. 262816) by the U.S. Environmental Protection Agency.

MRID NUMBER: 407983-04.

TEST MATERIAL: Thidiazuron.

SYNONYM(S)/CAS No.: Not listed.

SPONSOR: NOR-AM Chemical Co., Wilmington, DE.

TESTING FACILITY: NOR-AM Chemical Co., Wilmington, DE/Hazleton Laboratories America, Inc., Kensington, MD.

TITLE OF REPORT: Comments on the review of the mutagenicity evaluation of thidiazuron, Batch No. 7/9.82 in an *in vitro* cytogenic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures (T45 Thidiazuron, Accession No. 262816) by the U.S. Environmental Protection Agency.

AUTHOR(S): Chisholm, K.W.

STUDY NUMBER(S): Not applicable.

REPORT ISSUED: August 16, 1988.

A. SUMMARY:

An EPA toxicology review of an in vitro human lymphocyte cytogenetic study conducted with thidiazuron [Ivett, J. L., and Galloway, S. M. Mutagenicity evaluation of thidiazuron, batch No. 7/9.82 in an in vitro cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures. (Unpublished study No. LBI 20990 prepared by Litton Bionetics, Inc., Kensington, MD, for Schering AG, Berlin, FRG; dated June 1984.) Accession No. 262816.] was completed in July 1987.

Comments were received from the sponsor and the performing laboratory regarding the EPA review of this study. EPA reviewers considered the nonactivated portion of the assay unacceptable because the sensitivity of the system for detecting a direct-acting clastogen was questionable, and rare complex aberrations were seen at two assayed doses of the test material. However, the S9-activated assay was acceptable. We contend that the original conclusions were accurate and, therefore, do not change our position regarding the unacceptability of the nonactivated test results. Our responses to NOR-AM's and Hazleton's comments are discussed below:

B. RESPONSE TO COMMENTS:

1. Technical Problems: The nonactivated phase of the human lymphocyte cytogenetic assay with thidiazuron was rejected because the overall results raised doubts regarding the technical quality of the study. The review did not conclude that nonactivated thidiazuron was positive but that the negative results were equivocal. Several factors contributed to this assessment:

   - The nonactivated positive control (Mitomycin C) induced a weak positive effect. Although the effect was significant ($p < 0.05$), only four chromosome aberrations were observed (two quadriradials and two chromosome breaks). Additionally, twice the number of metaphases (50) had to be scored to achieve this borderline response as compared with the 25 metaphase plates analyzed for the S9-activated positive control. The usual approach taken by the performing laboratory (based on our review of numerous cytogenetic assays performed by Hazleton and submitted to EPA) is to count only 25 metaphases in the positive control groups. Thus, the counting of twice the routine number of cells suggested to our reviewers that a technical problem existed.

- Mitomycin C is a powerful clastogen; adverse effects on chromosomes can be detected after 3 hours of cell exposure. Although the dose used in this study was low (0.25 μg/mL), the length of exposure of the lymphocytes (50 hours) should have been sufficient to cause a highly significant effect. It is not certain, however, if increasing the dose would have resulted in a clear positive response under the conditions used.

- Positive control slides were not coded, thereby introducing an unacceptable level of bias.

- The sponsor and performing laboratory contend that the S9-activated positive control (cyclophosphamide) induced a strong positive response in the same donor cells; therefore, the overall sensitivity of the system was adequately demonstrated. In contrast to this position, EPA considers in vitro positive controls to serve a dual purpose: 1) the determination of cell line sensitivity to detect a positive effect; and 2) the determination of assay sensitivity under the specific conditions of a given experiment. Since there was no indication that both phases of the study were conducted simultaneously, the demonstration of a positive response with S9-activated cyclophosphamide is not relevant to the nonactivated assay findings. It is noteworthy that three S9-activated assays were performed with thidiazuron in order to achieve a single valid test. No data were provided from the initial assay and our reviewers rejected the data from the second assay because cyclophosphamide failed to induce a clastogenic effect. As these comments suggest, assay sensitivity was also a problem under the conditions of S9 activation. There is further a suggestion that the target cells were not uniformly affected by the test material owing to either poor solubility or culture variability.

2. Test Material Results: We agree with the sponsor and study author that nonactivated thidiazuron did not induce a significant increase in aberrations at any assayed dose. However, the presence of the same type of rare chromosome aberrations in the test groups (one quadriradial each at two test doses in comparison with two in the positive control group) remains; if this is not a cause for concern, at least it is an issue that should be resolved. The study author stated that quadriradials are rare events and have a background frequency in human lymphocytes of 1/1000 (0.1%); however, they failed to note that the frequency in the test groups (1/100 at two dose levels) was 1%, which is 10 times higher than background. We contend that this increase was suspiciously high and should have been

verified by performing a repeat assay to determine whether the occurrence of quadriradials in the test groups was a random event.

C. OVERALL CONCLUSIONS:

We concluded, based on a reevaluation of the data and consideration of the received comments, that our initial assessment of the nonactivated human lymphocyte assay with thidiazuron was correct. Until these issues are resolved, the nonactivated portion of this study will remain unacceptable. Therefore, our previous recommendation that the nonactivated assay be repeated is unchanged.

D. CBI APPENDIX:

Appendix A, Response to EPA Comments, CBI pp. 3-4; Appendix B, Data Evaluation Record 255-B: Ivett, J. L., and Galloway, S. M. Mutagenicity evaluation of thidiazuron, batch No. 7/9.82 in an in vitro cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures. (Unpublished study No. LBI 20990 prepared by Littor Bionetics, Inc., Kensington, MD, for Schering AG, Berlin, FRG; dated June 1984.) Accession No. 262816.

APPENDIX A

Response to EPA Comments
CBI pp. 3-4

APPENDIX B

Data Evaluation Record 255-B