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OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PREVENTION, PESTICIDES, AND TOXIC SUBSTANCES
WASHINGTON, D.C. 20460

December 9, 2004

MEMORANDUM

Subject: **Thidiazuron** - Reviews of Nine Toxicity Studies:

PC CODE: 120301 **DP BARCODE:** D294559, D305092
TXR#: 0052299

To: Stephanie Plummer/Susan Lewis
Reregistration Branch I
Special Review and Reregistration Division (7508C)

From: Paul Chin, Ph.D.
Reregistration Branch I
Health Effects Division (7509C)

Through: Whang Phang, Ph.D.
Senior Scientist
Reregistration Branch I
Health Effects Division (7509C)

The registrant, Aventis CropScience, submitted nine studies (see below). These studies were reviewed by the contractor, Dynamac Corporation and went through the secondary review process in HED. The DERs for these studies are attached to this memorandum. The following lists each study with the MRID number:

Acute Oral Toxicity Study in Rats (MRID 46121501)
Acute Dermal Toxicity Study in Rats (MRID 46121502)
Acute Inhalation Toxicity Study in Rats (MRID 46121503)
Dermal Sensitization Study (MRID 46121504)
90-Day Oral Toxicity Study in Mice (MRID 46121505)
Subchronic Oral Toxicity Study in Rats (MRIDs 46121506 and 46121509)
Developmental Toxicity Study in Rabbits (MRID 46121507; 46241001, 46252001)
Bacterial Reverse Gene Mutation Assay (MRID 46121508)

MAR 16 2005

In Vitro Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes (MRID 46121510)

In general, the above studies showed that acute oral, dermal and inhalation toxicity of thidiazuron to be in toxicity categories III and IV. Thidiazuron was not a dermal sensitizer. Subchronic toxicity studies in rats showed that thidiazuron caused decreased body weights, body weight gains, and food consumption and decreased epididymides and prostate gland weights, small prostate and small seminal vesicles. In addition, microscopic findings were observed in the prostate gland, seminal vesicle, mammary gland, thymus, the bone and marrow of the sternum and ovary, adrenal gland, kidney, liver, and spleen. The results of the subchronic feeding study in mice showed that thidiazuron produced increased incidences of centrilobular hepatocellular hypertrophy in the males and diffuse acinar hypertrophy in the salivary glands in the females.

In developmental toxicity study, maternal toxicity such as increased incidence of abortions and decreases in the body weight and food consumption were seen in rabbits at 125 mg/kg/day. At the same dose, developmental toxicity observed was decreased fetal weight, increased number of runts, and delayed skeletal ossification.

The standard battery of genotoxicity tests (Bacterial Reverse Gene Mutation Assay and *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes) were negative.

The MRID 46121500 is a correspondence from the registrant.

DATA EVALUATION RECORD

THIDIAZURON

Study Type: §81-1; Acute Oral Toxicity Study in Rats

Work Assignment No. 1-01-17 A (MRID 46121501)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticide Health Effects Group
Sciences Division
Dynamac Corporation
2275 Research Boulevard
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Primary Reviewer:
David McEwen, B.S.

Signature: David A. McEwen
Date: 3/9/04

Program Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 3/19/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature:** Paul Chi
Reregistration Branch 1, Health Effects Division (7509C) **Date** 9/28/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/29/04
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** Ghazi Dannan
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Oral Toxicity - Rat; OPPTS 870.1100 [§81-1]; OECD 401.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Coleman, D.G. (2001) Thidiazuron (Code: AE B049537): Rat acute oral toxicity. Huntingdon Life Sciences Limited, Huntingdon, UK. Laboratory Study ID: Tox 20138, April 5, 2001. MRID 46121501. Unpublished.

SPONSOR: Aventis CropScience SA, 355 rue Dostievski, Sophia-Antipodist Cedex, France

EXECUTIVE SUMMARY - In an acute oral toxicity study (MRID 46121501), 5 fasted, Sprague-Dawley CD rats/sex were given a single oral (gavage) dose (2000 mg/kg at a volume of 20 mL/kg) of Thidiazuron (98.7% a.i., Batch/Lot #: CH107623-02) in 1% methylcellulose and observed for 14 days.

Oral LD_{50} **Males >2000 mg/kg (observed)**
Females >2000 mg/kg (observed)
Combined > 2000 mg/kg (observed)

Thidiazuron is classified as **TOXICITY CATEGORY III** based on the oral LD_{50} observed in both sexes. No mortality was observed in either sex. There were no treatment-related necropsy findings. Clinical signs observed in 2000 mg/kg treated animals included the following: (i) lethargy and hunched posture in all animals beginning at 4-5 hours post dosing; (ii) deep respiration in 3/5 males and 4/5 females beginning at 5 hours post-dosing; (iii) piloerection in all animals beginning on Day 2; and (iv) abnormal gait in all females beginning on Day 2. All signs were resolved by Day 3. Body weight gain was decreased in 2/5 females between Days 8 and 15. No treatment-related effects on body weight were observed in the males.

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.1100; OECD 401) for an acute oral toxicity study in the rat.

COMPLIANCE - Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. **Test material:** Thidiazuron

Description:	Yellowish powder
Batch/Lot #:	CH107623-02
Purity (w/w):	98.7% a.i.
CAS # of TGAI:	51707-55-2

2. **Vehicle and/or positive control** - 1% (w/v) methylcellulose

3. **Test animals**

Species:	Rat
Strain:	Sprague-Dawley (Hsd:Sprague-Dawley (CD))
Age/weight at dosing:	8-11 weeks old/ 208-229 g males and 195-202 g females
Source:	Harlan U.K. Ltd., Bicester, Oxon, England
Housing:	Individually in suspended metal cages with grid floors
Diet:	Standard Laboratory Rodent Diet, RM1(E) SQC expanded pellet (Special Diet Services), *ad libitum*; except for overnight prior to dosing and for 4 hours post-dosing.
Water:	Tap water, *ad libitum*
Environmental Conditions	
Temperature:	22±3 °C
Humidity:	40-70%
Air changes:	Not reported
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	8 days

B. STUDY DESIGN and METHODS

1. **In-life dates** - Start: 10/31/2000 End: 11/24/2000

2. **Animal assignment and treatment** - Animals were randomly assigned to the test groups noted in Table 1. The test formulations were prepared on each day of dosing by mixing the test material in 1% methylcellulose at concentrations of 100 or 250 mg/mL for the 2000 and 5000 mg/kg doses, respectively. In the preliminary study, 1 rat/sex was dosed at 2000 or 5000 mg/kg

and then observed for 8 days following treatment. Based on the results of the preliminary study, 5 rats/sex were dosed at 2000 mg/kg and observed for 14 days following treatment. All rats were fasted overnight prior to being given a single dose of Thidiazuron by gavage (20 mL/kg), and food was withheld for approximately 4 hours post-dosing. The animals were observed twice daily for mortality, and were observed soon after dosing and at frequent intervals on Day 1, then twice daily thereafter for signs of toxicity. All animals were weighed on Days 1 (pre-dosing), 8, and 15. All surviving animals were sacrificed via carbon dioxide asphyxiation and subject to a necropsy on Day 15.

Table 1. Study design and mortality. [a]

Study Phase	Dose (mg/kg bw)	Mortality (number dead/number of animals)		
		Males	Females	Combined
Preliminary	5000	0/1	1/1	1/2
Preliminary	2000	0/1	0/1	0/2
Main	2000	0/5	0/5	0/10

a Data obtained from Study Report, page 13.

3. Statistics - Because there was no mortality in the main study, no LD_{50} calculation was performed.

II. RESULTS AND DISCUSSION

A. MORTALITY - In the preliminary study, the 5000 mg/kg female was found dead on Day 3. No mortality was observed at 2000 mg/kg in either the preliminary or main study (Table 1).

The oral LD_{50} for males is >2000 mg/kg
females is >2000 mg/kg

B. CLINICAL OBSERVATIONS - Clinical signs observed in 2000 mg/kg treated animals included the following: (i) lethargy and hunched posture in all animals beginning at 4-5 hours post dosing; (ii) deep respiration in 3/5 males and 4/5 females beginning at 5 hours post-dosing; (iii) piloerection in all animals beginning on Day 2; and (iv) abnormal gait in all females beginning on Day 2. All signs were resolved by Day 3.

C. BODY WEIGHT - Body weight gain was decreased in 2/5 females between Days 8 and 15. No treatment-related effects on body weight were observed in the males. Body weight and body weight gain historical control data were not provided.

D. NECROPSY - No treatment-related lesions were observed in any tissues examined during necropsy.

E. REVIEWER'S CONCLUSIONS - The reviewer agrees with the investigators that the oral LD_{50} is >2000 mg/kg.

F. DEFICIENCIES - The following minor deficiency was noted, but does not affect the results of this DER:

- Body weight and body weight gain historical control data were not provided.

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DATA EVALUATION RECORD

THIDIAZURON

Study Type: §81-2, Acute Dermal Toxicity

Work Assignment No. 1-01-17 B (MRID 46121502)

Prepared for
Health Effects Division
Office of Pesticide Programs
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Prepared by
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Primary Reviewer:
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Signature: David A. McEwen
Date: 3/8/04

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 3/19/04

Disclaimer

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EPA Reviewer: Paul Chin **Signature:** Paul Chi
Reregistration Branch 1, Health Effects Division (7509C) **Date** 9/28/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/29/04
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** Ghazi Dannan
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Dermal Toxicity - Rat; OPPTS 870.1200 [§81-2]; OECD 402.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Coleman, D.G. (2001) Thidiazuron: Rat acute dermal toxicity (Code: AE B049537). Huntingdon Life Sciences Limited, Huntingdon, UK. Laboratory Study ID: Tox 20139, April 5, 2001. MRID 46121502. Unpublished.

SPONSOR: Aventis CropScience SA, 355 rue Dostievski, F-06903 Sophia-Antipolis Cedex, France

EXECUTIVE SUMMARY - In an acute dermal toxicity study (MRID 46121502), groups of 5 Sprague-Dawley rats/sex were dermally exposed to Thidiazuron (98.7% a.i., Batch/Lot #: CH107623-02) in 1% (w/v) methylcellulose for 24 hours at a dose of 5000 mg/kg bw (limit dose). The test substance was applied to the intact skin in area covering approximately 10% of the total body surface. Animals were observed for clinical signs of toxicity and mortality for up to 14 days post-dosing.

Dermal LD_{50} Males >5000 mg/kg (observed)
Females >5000 mg/kg (observed)

Thidiazuron is considered to be of **LOW dermal toxicity** and is classified as **TOXICITY CATEGORY IV** based on the dermal LD_{50} observed in both sexes. No mortality was observed in either sex and there were no treatment related effects on clinical signs, or necropsy findings. Treatment-related systemic effects were limited to decreased body weight and body weight gains in females on Day 8. Dermal irritation was observed in 3/10 treated animals. These animals showed signs of slight erythema following removal of the dressing. The effect was resolved the following day.

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.1200; OECD 402) for an acute dermal toxicity study in the rat.

COMPLIANCE - Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description:	Yellowish powder
Batch/Lot #:	CH107623-02
Purity (w/w):	98.7% a.i.
CAS # of TGAI:	51707-55-2

2. Vehicle and/or positive control - 1% (w/v) methylcellulose

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley (Hsd:Sprague-Dawley (CD))
Age/weight at dosing:	8-11 weeks old/ 241-260 g males and 222-237 g females
Source:	Harlan U.K. Ltd., Bicester, Oxon, England
Housing:	Individually in suspended metal cages with grid floors
Diet:	Standard Laboratory Rodent Diet, RM1(E) SQC expanded pellet (Special Diet Services), *ad libitum*
Water:	Tap water, *ad libitum*
Environmental Conditions	
Temperature:	21±2 °C
Humidity:	40-70%
Air changes:	Not reported
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days

B. STUDY DESIGN and METHODS

1. In-life dates - Start: 11/02/2000 End: 11/16/2000

2. Animal assignment and treatment - Animals were assigned to the test group noted in Table 1. Approximately 24 hours prior to testing, the fur was clipped from the trunks of 5 Sprague-Dawley rats/sex. The appropriate amount of thidiazuron was mixed with 1% methylcellulose to provide a dose of 5000 mg/kg, and a volume of 20 mL/kg was applied to an area of the shaved intact skin (50 mm^2, approximately 10% of the total body surface area). The treatment area was

covered with porous gauze and held in place with a non-irritating dressing. This dressing was further wrapped with a waterproof dressing wrapped around the trunk of each animal. After a 24 hour exposure period, the dressings were removed, the test site washed with water, and the area was blotted dry. The animals were observed for twice daily for mortalities, and frequently on the day of dosing (Day 1) and twice daily thereafter for clinical signs. The test site was evaluated for dermal irritation (erythema and/or oedema) daily beginning on Day 2. All animals were weighed on Days 1 (prior to dosing), 8, and 15. On Day 15, all animals were sacrificed and a necropsy was performed.

Table 1. Study design and mortality. [a]

Dose (mg/kg bw)	Mortality (# of deaths/# treated animals)		
	Males	Females	Combined
5000	0/5	0/5	0/10

a Data obtained from Study Report page 13.

3. Statistics - Because there was no mortality, no LD_{50} calculation was performed.

II. RESULTS AND DISCUSSION

A. MORTALITY - No mortality was observed (Table 1).

The dermal LD_{50} for males is >5000 mg/kg bw
females is >5000 mg/kg bw
combined is >5000 mg/kg bw

B. CLINICAL OBSERVATIONS - It was stated that no clinical signs were observed in any animal during the 14-day observation period; however, no tabular data were provided. Slight erythema was noted in 2/5 males and 1/5 females on Day 2, and it was resolved by Day 3 in all cases. Additionally, one male displayed localized spots/scabbing on Days 3-5.

C. BODY WEIGHT - In the females, group mean body weight gain was 0 g on Day 8 compared to Day 1. This finding was mainly due to body weight losses of 6 g each in two females and zero gain in one other female. All but one female showed body weight gain by Day 15. Historical control data were not provided.

D. NECROPSY - No gross abnormalities were observed during necropsy in either sex.

E. REVIEWER'S CONCLUSIONS - No mortality was observed. No treatment-related effects on clinical signs, dermal irritation, or gross pathology were observed in either sex at 5000 mg/kg (limit dose). Treatment-related effects were limited to decreased body weight and body weight gains in females on Day 8.

F. DEFICIENCIES - The following minor deficiencies were noted, but would not change the conclusions of this review:

- Clinical signs tabular data were not provided.
- Historical control body weight data were not provided.

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DATA EVALUATION RECORD

THIDIAZURON

Study Type: §81-3; Acute Inhalation Toxicity

Work Assignment No. 1-01-17 C (MRID 46121503)

Prepared for
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Office of Pesticide Programs
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Primary Reviewer:
David A. McEwen, B.S.

Signature: David A. McEwen
Date: 3/9/04

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 3/19/04

Disclaimer

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EPA Reviewer: Paul Chin **Signature**: Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 9/28/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/29/05
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** Shah
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Acute Inhalation Toxicity - Rat; OPPTS 870.1300 [§81-3]; OECD 403.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Wesson, C.M (2001) Thidiazuron (AE B049537): Acute inhalation toxicity (nose only) study in the rat. Safepharm Laboratories Limited, Derby, UK. Laboratory Study ID: 374/096, March 29, 2001. MRID 46121503. Unpublished.

SPONSOR: Aventis CropScience SA, 355 rue Dostievski, Sophia-Antipolis Cedex, France

EXECUTIVE SUMMARY - In an acute inhalation toxicity study (MRID 46121503), young adult Sprague-Dawley rats (5/sex) were exposed by nose-only inhalation to Thidiazuron (aerosol) (98.7% a.i., Batch/Lot #: CH107623-02) for 4 hours at a gravimetrically determined concentration of 3.38 mg/L. The animals were observed for up to 14 days post-exposure.

Inhalation LC_{50} Males > 3.48 mg/L
Females > 3.48 mg/L

No mortality occurred in this limit test. Thidiazuron is considered to be of **LOW Toxicity** and is classified as **TOXICITY CATEGORY IV** based on the LC_{50} in both sexes. There were no treatment related necropsy findings or changes in body weight. Increased and/or noisy respiration was observed during exposure and through Day 2.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.1300; OECD 403) for an acute inhalation toxicity study in the rat.

COMPLIANCE - Signed and dated GLP, Data Confidentiality, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS

1. Test material: Thidiazuron

Description:	Beige solid
Batch/Lot #:	CH107623-02
Purity (w/w):	98.7% a.i.
CAS # of TGAI:	51707-55-2

2. Vehicle - Air

3. Test animals

Species:	Rat
Strain:	Sprague-Dawley (Crl:CD® (SD) IGS BR)
Age/weight at dosing:	Approximately 8-10 weeks old/ 280-318 g males and 219-239 g females
Source:	Charles River (UK) Ltd., Margate, Kent, England
Housing:	In groups of 5/sex in solid floor polypropylene cages with stainless steel lids
Diet:	Rat and Mouse Expanded Diet No. 1 (Special Diet Services Ltd., Witham, Essex, UK), *ad libitum*; except during the exposure period
Water:	Tap water, *ad libitum*; except during the exposure period
Environmental Conditions	
Temperature:	21±2 °C
Humidity:	55±15%
Air changes:	At least 15/Hr
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	at least 5 days

B. STUDY DESIGN and METHODS

1. In-life dates - Start: 11/01/2000 End: 01/19/2001

2. Exposure conditions

a. Animal assignment and treatment - Animals were assigned to the test groups noted in Table 1. Rats were exposed to Thidiazuron by nose-only exposure for 4 hours. All animals were observed for clinical signs hourly during exposure, immediately upon removal of restraining tubes, one hour post-exposure, and once daily thereafter during the 14-day observation period. All animals were weighed on Days 0 (prior to exposure), 7, and 14. All surviving animals were sacrificed on Day 14 and a gross necropsy was performed. The respiratory tract was subjected to a detailed macroscopic examination for signs of irritation or local toxicity.

Table 1. Study design and mortality.[a]

Nominal Conc. (mg/L)	Analytical Conc. (mg/L)	MMAD (µm)	Inhalable Fraction (% <4 µm)	GSD	Mortality (# dead/total)		
					Males	Females	Combined
22.0	3.48	3.42	56.5	2.58	0/5	0/5	0/10

a Data were obtained from pages 16-17 of the study report.

b. Generation of the test atmosphere/chamber description - A 30 L nose-only chamber (ADG Developments Ltd., Hitchin, Herts, UK) was used for this study. The chamber temperature was 19-21°C, and the relative humidity was 30-55%. The airflow was maintained at approximately 18 L/min, and the atmosphere contained 20.5-20.6% oxygen. The time to equilibrium (t_{99}) was 8 minutes.

The test material was converted to an aerosol using a Wright's Dust Feed mechanism (L. Adams Ltd., London, UK). Chamber airflow, temperature, and relative humidity were recorded every 30 minutes during the 4-hour exposure period.

The test atmosphere concentration was determined gravimetrically five times during the 4-hour exposure period by taking samples (1 L) from the animals breathing zone. The aerosol particles were collected on glass fibre filters (Gelman type A/E 25 mm) and the time-weighted average concentration was calculated from the gravimetric measurements (Table 1). In addition, particle size was determined three times during the exposure period by drawing samples (of a suitable known volume) from the animals breathing zone through a six-stage Marple Personal Cascade Impactor (Schaefer Instruments Ltd., Oxon, UK). The Mass Median Aerodynamic Diameter (MMAD) and geometric standard deviation (GSD) were then determined. Additionally, the proportion (%) of aerosol less than 4 µm was determined.

3. Statistics - Because there was no mortality, a LC_{50} calculation was not performed.

II. RESULTS and DISCUSSION

A. MORTALITY - No mortality occurred during the study (Table 1).

The inhalation LC_{50} for males is >3.48 mg/L
females is >3.48 mg/L

B. CLINICAL OBSERVATIONS - During exposure, increased respiratory rate and wet fur were observed in all animals. After removal from the chamber, all animals displayed increased respiratory rate, hunched posture, piloerection, and wet fur. Pallor of the extremities, noisy respiration, isolated incidences of red/brown staining around the eyes, and sneezing were also observed following removal from the chamber. All animals appeared normal by Day 3. It was stated that the wet fur, hunched posture, piloerection, and red/brown staining around the eyes are commonly seen in rats for short periods following 4-hour exposures, and are considered to be

associated with the restraint procedure, and are not indicative of toxicity.

C. BODY WEIGHT - All animals showed normal weight gains during the 14-day observation period.

D. NECROPSY - No treatment-related gross lesions were observed during necropsy.

E. REVIEWER'S CONCLUSIONS - The reviewer agrees with the study author that there were no treatment related necropsy findings, or changes in body weight. Increased and/or noisy respiration was observed during exposure and through Day 2. Thidiazuron is considered to be of **LOW Toxicity** and is classified as **TOXICITY CATEGORY IV** based on the LC_{50} in both sexes.

F. DEFICIENCIES - None

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DATA EVALUATION RECORD

THIDIAZURON

Study Type: §81-6; Dermal Sensitization

Work Assignment No. 1-01-17 D (MRID 46121504)

Prepared for
Health Effects Division
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Signature: David A. McEwen
Date: 3/8/04

Project Manager:
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 3/18/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

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Reregistration Branch 1, Health Effects Division (7509C) **Date** 6/1/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/27/04
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** Shah Dawson
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Skin Sensitization - Guinea Pig; OPPTS 870.2600 [§81-6]; OECD 406.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Coleman, D.G. (2001) Thidiazuron (Code: AE B049537): Guinea Pig skin sensitization study. Huntingdon Life Sciences Limited, Huntingdon, UK. Laboratory Study ID: Tox 20140, April 5, 2001. MRID 46121504. Unpublished.

SPONSOR: Aventis CropScience SA, 355 rue Dostievski, F-06903 Sophia-Antipolis Cedex, France

EXECUTIVE SUMMARY - In a dermal sensitization study (MRID 46121504), 20 female Dunkin-Hartley guinea pigs were exposed to Thidiazuron (98.7% a.i., Batch/lot #: CH107623-02) in Alembicol D or a 50:50 solution of Alembicol D and Freund's Complete Adjuvant (FCA), using the Magnusson-Kligman Maximization test. An additional 10 females were used for controls. Positive control data were submitted for hexylcinnamic aldehyde.

No dermal reactions were observed in any animal during the challenge phase. **Under the conditions of this study, Thidiazuron is not a dermal sensitizer.** No treatment-related effects on clinical signs or body weight were observed.

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.2600; OECD 406) for a dermal sensitization study in the guinea pig.

COMPLIANCE - Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description:	Off-white powder
Batch/Lot #:	CH107623-02
Purity (w/w):	98.7% a.i.
CAS # of TGAI:	51707-55-2

2. Vehicle and/or positive control - Freund's Complete Adjuvant (FCA) and Alembicol D; in a separate study conducted within 5 months of the current study, hexylcinnamic aldehyde served as the positive control.

3. Test animals

Species:	Guinea pig
Strain:	Dunkin/Hartley
Age/weight at dosing:	5-8 weeks old/ 308-379 g females only
Source:	D. Hall, Newchurch, Staffs, UK
Housing:	Groups of 5 in suspended plastic cages with solid floors
Diet:	Vitamin C enriched guinea pig diet (Harlan Teklad 9600 FD2 SQC), *ad libitum*. Hay was supplied three times per week.
Water:	Tap water, *ad libitum*
Environmental Conditions	
Temperature:	21±3 °C
Humidity:	30-70%
Air changes:	Not reported
Photoperiod:	12 hrs dark/12 hrs light
Acclimation period:	7 days

B. STUDY DESIGN and METHODS

1. In-life dates - Start: 12/04/2000 End: 01/15/2001

2. Animal assignment and treatment - The Magnusson-Kligman Maximization method was used in this study. A preliminary screening study, consisting of two phases, was performed to establish the highest non-irritating dose of the test material. In phase one, the hair was clipped/shaved from the flanks of 2 animals prior to test article administration. Each animal received intradermal injections (0.1 mL each) of the test material in Alembicol D at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0, 7.5, or 10 % (w/v). The injection sites were observed and scored for dermal irritation at 24 and 72 hours post-dosing. In phase 2, four animals received a single intradermal injection of a 50:50 FCA and water solution approximately

1 week prior to topical induction. Approximately 24 hours prior to test article administration, the hair was clipped/shaved from both flanks of all animals. Filter papers (2x2 cm Whatman No. 3) were loaded with approximately 0.2 mL of the test material in Alembicol D at 10, 20, 40, or 60% (w/v) and applied to the test areas (2/flank) of each animal. Each patch was covered with an occlusive dressing (Blenderm and Elastoplast) for 24 hours. The sites were observed and scored for signs of dermal irritation at 24 hours post-dosing, and 24 and 48 hours after removal of the dressings. Based on the results of the preliminary study, the following concentrations of Thidiazuron were chosen for the main study: 7.5% (w/v; intradermal injection), 60% (w/v; topical induction), and 30 and 60% (w/v; topical challenge).

In the induction phase, fur was clipped from a 4x6 cm area on the scapular region of 30 guinea pigs (20 in the treatment group and 10 controls) approximately 24 hours prior to treatment. On Day 1, three pairs of intradermal injections (0.1 mL) were made within a 2x4 cm test area such that there were two parallel lines of three injections each on either side of the spinal column. The injections for all animals were as follows: 50:50 FCA and water, 7.5% Thidiazuron in Alembicol D, and 7.5% Thidiazuron in a 50:50 solution of Alembicol D and FCA. The injection sites were examined for signs of dermal irritation at 24 hours post-dosing. On Day 7, the clipped dorsa of all animals were treated with 10% (w/w) sodium lauryl sulphate in petrolatum (0.5 mL) to enhance dermal absorption for the following day. On Day 8, filter papers (2x4 cm Whatman No. 3) were loaded with 0.4 mL of Thidiazuron in Alembicol D at 60% (w/v) and applied over the injection sites of the test animals. Control animals were similarly treated using Alembicol D only. Each patch was covered with an occlusive dressing (Blenderm and Elastoplast). The patches were removed after 48 hours and the test sites were observed for signs of dermal irritation.

In the challenge phase, the left flanks of all animals were clipped/shaved on Day 22. Absorbent patches (2x2 cm) were loaded with 0.2 mL of Thidiazuron in Alembicol D at 30 or 60% (w/v) and applied to the anterior (60%) and posterior (30%) of the left flank of each animal. Each patch was covered with an occlusive dressing (Blenderm and Elastoplast) for 24 hours. After the exposure period, the dressings were removed and examined for dermal irritation at 24 and 48 hours after patch removal. The degree of dermal reaction (erythema or oedema) was graded using the following five point scale:

Grade	Reaction to treatment
0	No response
1	Slight erythema/oedema
2	Well defined erythema/oedema
3	Moderate erythema/oedema
4	Severe erythema/oedema

All animals were observed twice daily for clinical signs of toxicity, and were weighed prior to the first treatment and at termination.

II. RESULTS and DISCUSSION

A. INDUCTION REACTIONS AND DURATION - Necrosis was observed at all sites receiving FCA in the treated and control animals. Slight irritation was observed in all test animals at sites receiving 7.5% (w/v) Thidiazuron in Alembicol D and in all control animals receiving Alembicol D. No erythema was observed in any animal following topical application of 60% (w/v) Thidiazuron.

B. CHALLENGE REACTIONS AND DURATION - No dermal reactions were observed in any animal during the challenge phase. **Under the conditions of this study, Thidiazuron is not a dermal sensitizer.** No treatment-related effects on clinical signs or body weight were observed.

C. POSITIVE CONTROL - Positive control data were provided from a study conducted within 5 months of the current study. The results of the dermal sensitization study using hexylcinnamic aldehyde indicate the test method's ability to detect a dermal sensitizer.

D. REVIEWER'S CONCLUSIONS - The reviewer agrees with the investigators that under the conditions of this study, Thidiazuron is not a dermal sensitizer in guinea pigs.

E. DEFICIENCIES - None

6

DATA EVALUATION RECORD

THIDIAZURON

Study Type: §82-1b, 90-Day Oral Toxicity Study in Mice

Work Assignment No. 1-01-17 E (MRID 46121505)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by
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Program Manager Mary L. Menetrez, Ph.D.	Signature: Mary L Menetrez Date: 03-16-04
Quality Assurance Steve Brecher, Ph.D.	Signature: Steven Brecher Date: 3/16/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 10/26/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** WPhang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 10/28/04
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** Daniels
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04

Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity in Mice (diet); OPPTS 870.3100 [§82-1b]; OECD 408.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** Not provided

TEST MATERIAL (PURITY): Thidiazuron technical (99.5% a.i.)

SYNONYMS: *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea

CITATION: Foulon, O. (2001) Thidiazuron: 90-day toxicity study in the mouse by dietary administration. Aventis CropScience, 355, rue Dostoïevski, Sophia Antipolis Cedex, France. Laboratory Report of Study SA 00593, November 26, 2001. MRID 46121505. Unpublished.

SPONSOR: Aventis CropScience, 14-20, rue Pierre Baizet, Lyon Cedex, France

EXECUTIVE SUMMARY - In a subchronic oral toxicity study (MRID 46121505), Thidiazuron (99.5% a.i., Batch/Lot #: 107623-03) was administered to 10 C57BL/6JICO mice/sex/dose in the diet at doses of 0, 500, 1000, 2000, or 4000 ppm (equivalent to 0/0, 85.2/99.8, 170.9/202.6, or 351.4/383.9 mg/kg/day [M/F]) for up to 90 days.

Urinalysis, hematology, neurological evaluations, and ophthalmoscopic examinations were not performed. There were no treatment-related macroscopic findings.

All mice in the 4000 ppm group died or were sacrificed moribund on Days 6-9. In this group, animals exhibited the following clinical signs of toxicity prior to their death or sacrifice: (i) reduced motor activity, prostration, piloerection, dyspnea, cold to touch, thin appearance, and hunched posture in both sexes; (ii) staggering step in males; and (iii) no feces in females. Body weights were decreased by 28-29% ($p<=0.001$) in the two males and three females still alive on Day 8. Food consumption was decreased by 53-62% ($p<=0.001$) in both sexes for Days 1-8.

At 2000 ppm, hunched posture was observed in two males and one female on Day 15, and reduced motor activity was noted in one female on Days 28-29. Body weights were decreased by 4-7% ($p<=0.05$) throughout treatment in the males, except Days 57 and 90, and throughout treatment by 6-17% in the females. Overall (Days 1-90) body weight gains at this dose were decreased in the males (decr. 19%; not significant) and females (decr. 40%; $p<=0.001$). Weekly food consumption was decreased throughout treatment in the females (decr. 7-23%), resulting in decreased average food consumption for the overall (Days 1-90) study (decr. 12%; $p<=0.01$). Additionally in the 2000 ppm males, alkaline phosphatase was increased by 14% ($p<=0.05$), and albumin was decreased by 9% ($p<=0.05$). At 2000 ppm in both sexes, terminal body weights were decreased by 5-9% ($p<=0.05$), and relative (to body) liver weights were increased by 10-16% ($p<=0.01$). Slight centrilobular hepatocellular hypertrophy was observed in the 2000 ppm females (2/10 treated vs 0/10 controls).

At >=1000 ppm in the males, cholesterol was dose-dependently decreased by 16-34% ($p<=0.05$) and absolute, relative (to body), and relative (to brain) kidney weights were decreased by 9-15% ($p<=0.01$). The organ weight changes in the kidney were considered equivocal because there were no macroscopic or microscopic findings in the kidney. Additionally, slight centrilobular hepatocellular hypertrophy was observed in the >=1000 ppm males (1-9/10 treated vs 0/10 controls). Increased incidences of slight diffuse acinar hypertrophy were observed in the submaxillary salivary glands of the >=1000 ppm females (4-8/10 treated vs 1/10 controls).

At 500 ppm, no treatment-related effects were found.

The LOAEL is 1000 ppm (equivalent to 170.9/202.6 mg/kg/day in M/F) based on decreased cholesterol in the males and on increased incidences of centrilobular hepatocellular hypertrophy in the males and diffuse acinar hypertrophy in the salivary glands in the females. The NOAEL is 500 ppm (equivalent to 85.2/99.8 mg/kg/day in M/F).

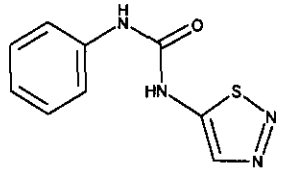
This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3100; OECD 408) for a 90-day oral toxicity study in the mouse.

COMPLIANCE - Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description:	Light yellow powder
Batch/Lot #:	107623-03
Purity (w/w):	99.5% a.i.
Stability of compound:	The test material was stable in the diet for up to 10 weeks at room temperature or 9 weeks at -15°C followed by one week at room temperature.
CAS #:	51707-55-2
Structure:	

2. Vehicle - Diet

3. Test animals

Species:	Mouse
Strain:	C57BL/6JICO
Age/weight at initiation of treatment:	6-7 weeks old; 15.7-21.5 g males, 13.8-18.4 g females
Source:	Iffa-Credo, France
Housing:	Individually in suspended stainless steel wire mesh cages
Diet:	Certified rodent irradiated and powdered diet AO4C-10P1 (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*, except for fasting prior to blood collection.
Water:	Filtered and softened tap water, *ad libitum*
Environmental conditions	
Temperature:	20-24°C
Humidity:	40-70%
Air changes:	Approximately 15/hr
Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	13 days

B. STUDY DESIGN

1. In life dates - Start: 01/17/01 End: 04/20/01

2. Animal assignment - An automatic procedure was used to select animals for the study from the middle of the weight range, so that the weight of each animal selected was within ±20% of

the mean weight for each sex. These animals were randomly assigned, stratified by body weight, to the test groups shown in Table 1.

Table 1. Study design [a]

Test Group	Dose (ppm)	Achieved Intake (mg/kg/day, M/F)	# of Animals (M/F)
Control	0	0/0	10/10
Low	500	85.2/99.8	10/10
Mid	1000	170.9/202.6	10/10
Mid-high	2000	351.4/383.9	10/10
High	4000	NA	10/10

a Data were obtained from pages 15, 51, and 55 of the study report.

NA Not available because all animals in this group were found dead or sacrificed moribund on or before Day 9.

3. Dose selection rationale - In a dose range-finding study, 5 mice/sex were intended to be fed Thidiazuron (99.5% a.i.; Batch #107623-03) in the diet at 5400 ppm for 10 days. Body weights were recorded on Days 1 and 5, and food consumption was recorded during the same period. All animals died or were found moribund from Day 5 to 7 in the females and from Day 7 to 9 in the males. Low food consumption was noted for both sexes, and a body weight loss was observed for all animals from Days 1-5 (-6.1 g for the females and -6.3 g for the males). No further information was provided.

4. Treatment preparation, administration, and analysis - The appropriate amount of test substance was ground to a fine powder before being incorporated into the diet by dry mixing to provide the required concentrations. Diet formulations were prepared once for the 4000 ppm diet and twice during the study for the 500, 1000, and 2000 ppm diets. When not in use, the test diets were stored at -15°C. Homogeneity of the test substance in the diet was verified at 500 and 4000 ppm from the first formulations prepared for the study. Stability of the test substance in the diet was confirmed in a trial mix for up to 10 weeks at room temperature or up to 9 weeks at -15°C followed by one week at room temperature. Concentration was analyzed for each dose level in each preparation.

Results - **Homogeneity (range as % nominal):** 88-113%

Stability (range as % nominal): 93-113%

Concentration (range as % nominal): 94-107%

The analytical data indicated that the mixing procedure was adequate and the variation between nominal and actual dosage to the animals was acceptable.

6. Statistics - The following statistical procedures were employed:

Parameter	Statistical Test
Body weights Body weight gains Food consumption Clinical chemistry Quantitative urinalyses parameters Terminal body weights Organ weights	Levene's test for homogeneity of variance. If variances were homogeneous, data were analyzed using analysis of variance (ANOVA) followed by pair-wise comparison of treated groups with the control group via Dunnett's test, if significant ANOVA. If variances were not homogeneous, data were analyzed using robust linear regression methods. Wald chi-square tests were used to test for an effect of treatment among all groups, followed by pair-wise comparison of treated groups with the control group via t-tests, if the Wald chi-square test was significant.

Significance was defined at $p \leq 0.05$. All tests were two-sided. The statistical methods were considered appropriate.

C. METHODS

1. Observations

a. Cage-side observations - Animals were inspected at least once daily for clinical signs of toxicity. All animals were checked for moribundity and mortality twice daily on weekdays and once daily on weekends and holidays.

b. Clinical examinations - Detailed physical examinations were performed prior to the start of administration and weekly thereafter.

c. Neurological evaluations - Not performed.

2. Body weight - All animals were weighed prior to the study, at initiation of treatment, weekly throughout the study, and at termination. Body weight gains were reported for various intervals throughout the study and for the overall (Days 1-90) study.

3. Food consumption and compound intake - For each animal, food consumption (g/animal/day) was determined weekly throughout the study and for the overall study. Test substance intake (mg/kg bw/day) was calculated for each week and for the overall study. Food efficiency was not reported.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were not performed.

5. Hematology and clinical chemistry - At termination (Days 91-94), blood samples were taken from the retroorbital venous plexus of all surviving animals. Mice were fasted overnight and

were anesthetized by inhalation of isoflurane prior to blood sampling. The following CHECKED (X) parameters were examined.

a. Hematology - No hematology parameters were evaluated.

b. Clinical chemistry

	ELECTROLYTES		OTHER
	Calcium*	X	Albumin*
	Chloride*		Creatinine*
	Magnesium	X	Urea nitrogen*
	Phosphorus*	X	Total Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine amino-transferase (also SGPT)*		
X	Aspartate amino-transferase (also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. Urinalysis - Urinalysis was not performed, and is not required by the Guidelines 870.3100.

7. Sacrifice and pathology - At study termination, all surviving animals were sacrificed via exsanguination under pentobarbital anesthesia after overnight fasting. All animals, including those found dead or sacrificed moribund, were subjected to a gross necropsy. However, macroscopic findings were not reported for animals in the 4000 ppm group because these mice died or were sacrificed moribund during Days 6-9. The following CHECKED (X) tissues from all animals (except for the 4000 ppm group) were collected, processed routinely, and stained with hematoxylin and eosin. Additionally, the (XX) organs were weighed from animals sacrificed on schedule.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (with optic nerves)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*			XX	Adrenal glands*+
X	Cecum*		UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder (not rat)*	X	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	X	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus (with cervix)*+	X	Joint (femur/tibia)
X	Lungs*	X	Mammary gland*	X	Harderian gland
	Nasal structures*	X	Vagina		
	Pharynx*				
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weight required for rodent studies

Histopathological examinations were performed on all the above-listed tissues from all the animals in the control and 2000 ppm groups. Additionally in the intermediate (50 and 1000 ppm) dose groups, the following were examined microscopically: (i) all tissues from the decedents; (ii) the liver, lung, kidney, and thyroid; (iii) the submaxillary salivary glands in the females; and (iv) all gross lesions and masses.

II. RESULTS

A. OBSERVATIONS

1. Mortality - All mice in the 4000 ppm group died or were sacrificed moribund on Days 6-9. One 2000 ppm male was sacrificed on Day 20 after accidental trauma. One 1000 ppm female was found dead on Day 51. No adverse effects on body weight, food consumption, or clinical

observations were observed in this animal prior to death; however, a brain hemorrhage observed at necropsy indicated accidental trauma. All other animals survived to scheduled sacrifice.

2. Clinical signs of toxicity - Prior to their death or sacrifice, animals in the 4000 ppm group exhibited the following clinical signs of toxicity (Table 2): (i) reduced motor activity, prostration, piloerection, dyspnea, cold to touch, thin appearance, and hunched posture in both sexes; (ii) staggering step in males; and (iii) no feces in females.

At 2000 ppm, hunched posture was observed in two males and one female on Day 15, and reduced motor activity was noted in one female on Days 28-29. No clinical signs of toxicity were observed at 500 or 1000 ppm.

Table 2. Clinical signs of toxicity (#animals affected) in mice treated with Thidiazuron in the diet for up to 90 days [a]

Clinical sign	Dose (ppm)				
	0	500	1000	2000	4000 [b]
Males					
Reduced motor activity	0	0	0	0	10
Prostration	0	0	0	0	5
Piloerection	0	0	0	0	2
Dyspnea	0	0	0	0	2
Cold to touch	0	0	0	0	1
Appears thin	0	0	0	0	6
Staggering step	0	0	0	0	3
Hunched posture	0	1	0	2	6
Females					
Reduced motor activity	0	0	0	1	9
Prostration	0	0	0	0	4
Piloerection	0	0	0	0	2
No feces	0	0	0	0	1
Dyspnea	0	0	0	0	3
Cold to touch	0	0	0	0	2
Appears thin	0	0	1	1	7
Hunched posture	0	0	0	1	8

a Data were obtained from Table 1 on pages 37-39 of the study report; n=10.
b All mice in the 4000 ppm group died or were sacrificed moribund on Days 6-9.

B. BODY WEIGHT AND WEIGHT GAIN - At 4000 ppm, body weights were severely decreased (28-29%; $p \leq 0.001$) in the two males and three females still alive on Day 8.

At 2000 ppm, body weights were decreased ($p \leq 0.05$ or $p <= 0.001$) throughout treatment in the males (↓4-7%) and in the females (↓6-17%; Table 3). Overall (Days 1-90) body weight gains at this dose were decreased in the males (↓19%; not significant) and females (↓40%; $p \leq 0.001$). There were no treatment-related differences in body weights or body weight gains in either sex at 500 or 1000 ppm.

Table 3. Selected mean (±SD) body weights and overall (Days 1-90) body weight gains (g) in mice treated with Thidiazuron in the diet for up to 90 days [a]

Study Day	Dose (ppm)			
	0	500	1000	2000
Males				
1	19.32 ± 1.57	19.35 ± 1.00	18.93 ± 1.17	19.24 ± 0.97
8	20.66 ± 1.02	20.54 ± 0.70	20.54 ± 1.68	19.71 ± 0.60* (↓5)
15	21.68 ± 0.95	21.58 ± 0.85	21.36 ± 1.92	20.23 ± 0.56*** (↓7)
50	24.12 ± 1.19	24.07 ± 0.84	23.61 ± 1.79	23.13 ± 0.76* (↓4)
85	25.92 ± 1.44	25.60 ± 1.24	24.93 ± 1.86	24.72 ± 0.69* (↓5)
Overall (Days 1-90) weight gain	6.95 ± 1.31	6.48 ± 1.03	6.32 ± 1.29	5.66 ± 0.79 (↓19)
Females				
1	16.65 ± 1.11	17.06 ± 0.78	16.67 ± 1.00	17.17 ± 0.75
8	17.62 ± 0.75	18.03 ± 0.99	17.38 ± 1.01	16.46 ± 1.11* (↓7)
36	20.43 ± 1.34	19.97 ± 1.24	19.79 ± 0.90	16.91 ± 1.03*** (↓17)
71	21.85 ± 1.14	21.78 ± 0.89	21.90 ± 0.69	20.35 ± 0.94** (↓6)
90	22.48 ± 1.15	22.31 ± 0.95	22.58 ± 0.64	20.67 ± 0.98*** (↓8)
Overall (Days 1-90) weight gain	5.83 ± 1.28	5.25 ± 0.99	5.93 ± 1.31	3.50 ± 0.70*** (↓40)

a Data were obtained from Table 2 on pages 41-46 of the study report; n=9-10. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from controls at $p \leq 0.05$

** Significantly different from controls at $p \leq 0.01$

*** Significantly different from controls at $p \leq 0.001$

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption - At 4000 ppm, food consumption was severely decreased (↓53-62%; $p \leq 0.001$) in both sexes for Days 1-8. Weekly food consumption was decreased throughout treatment in the 2000 ppm females (↓7-23%), resulting in decreased (↓12%; $p \leq 0.01$) average food consumption for the overall (Days 1-90) study (Table 4). No treatment-related effects on food consumption were observed in any other group in either sex.

Table 4. Selected mean (±SD) food consumption (g/animal/day) in female mice treated with Thidiazuron in the diet for up to 90 days [a]

Study Days	Dose (ppm)			
	0	500	1000	2000
15-22	4.0 ± 0.3	4.0 ± 0.3	3.8 ± 0.3	3.1 ± 0.2*** (↓23)
78-85	4.4 ± 0.4	4.6 ± 0.4	4.7 ± 0.5	4.1 ± 0.5 (↓7)
1-90	4.1 ± 0.3	4.2 ± 0.2	4.1 ± 0.3	3.6 ± 0.3** (↓12)

a Data were obtained from Table 3 on pages 52-53 of the study report; n=4-10. Percent difference from controls, calculated by the reviewers, is included in parentheses.
** Significantly different from the control group at $p \leq 0.01$
*** Significantly different from the control group at $p \leq 0.001$

2. Compound intake - Mean test material intake values for the overall study are reported in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION - Ophthalmoscopic examinations were not performed.

E. BLOOD ANALYSES

1. Hematology - No hematology parameters were evaluated.

2. Clinical chemistry - In the males, cholesterol was dose-dependently decreased at ≥1000 ppm (↓16-34%; $p \leq 0.05$; Table 5). Additionally at 2000 ppm, alkaline phosphatase was increased (↑14%; $p \leq 0.05$), and albumin was decreased (↓9%; $p \leq 0.05$). There were no other treatment-related differences in clinical chemistry at any dose in either sex.

Table 5. Selected mean (±SD) clinical chemistry parameters in male mice treated with Thidiazuron in the diet for 90 days [a]

Parameter	Dose (ppm)			
	0	500	1000	2000
Cholesterol (mmol/L)	1.516 ± 0.216	1.428 ± 0.182	1.272 ± 0.198* (↓16)	0.998 ± 0.180*** (↓34)
Alkaline phosphatase (IU/L)	64.2 ± 8.4	62.0 ± 6.7	65.2 ± 8.5	73.3 ± 8.1* (↑14)
Albumin (g/L)	36.8 ± 1.4	36.4 ± 2.8	34.4 ± 3.2	33.6 ± 2.8* (↓9)

a Data were obtained from Table 4 on page 57 of the study report; n=9-10. Percent difference from controls, calculated by the reviewers, is included in parentheses.
* Significantly different from the control group at $p \leq 0.05$
*** Significantly different from the control group at $p \leq 0.001$

F. URINALYSIS - Urinalysis was not performed.

G. SACRIFICE AND PATHOLOGY

1. Organ weight - In the 2000 ppm males and females, terminal body weights were decreased (↓5-9%; p≤0.05), and relative (to body) liver weights were increased (↑10-16%; p≤0.01; Table 6). These findings were considered treatment-related.

In the ≥1000 ppm males, absolute, relative (to body), and relative (to brain) kidney weights were decreased (↓9-15%; p≤0.01). Absolute kidney weights were decreased in the 2000 ppm females (↓10%; p≤0.05). The organ weight changes in the kidney were considered equivocal because there were no macroscopic or microscopic findings indicating a treatment-related effect.

Table 6. Selected mean (±SD) organ weights in mice treated with Thidiazuron in the diet for 90 days [a]

Parameter		Dose (ppm)			
		0	500	1000	2000
Males					
Terminal body weight (g)		22.19 ± 1.46	21.74 ± 1.01	21.26 ± 1.60	20.99 ± 0.64* (↓5)
Liver -	absolute (g)	0.90 ± 0.09	0.93 ± 0.07	0.88 ± 0.10	0.93 ± 0.07
	relative to body (%)	4.05 ± 0.23	4.28 ± 0.22	4.14 ± 0.36	4.44 ± 0.25** (↑10)
	relative to brain (%)	202.30 ± 20.18	210.46 ± 14.91	201.10 ± 19.81	213.77 ± 15.85
Kidney -	absolute (g)	0.329 ± 0.031	0.308 ± 0.016	0.286 ± 0.032** (↓13)	0.280 ± 0.007*** (↓15)
	relative to body (%)	1.482 ± 0.075	1.417 ± 0.053	1.343 ± 0.070*** (↓9)	1.335 ± 0.046*** (↓10)
	relative to brain (%)	73.959 ± 6.704	69.707 ± 3.705	65.379 ± 6.195** (↓12)	64.167 ± 2.421*** (↓13)
Females					
Terminal body weight (g)		18.50 ± 1.02	18.27 ± 0.78	18.56 ± 0.37	16.88 ± 0.80*** (↓9)
Liver -	absolute (g)	0.79 ± 0.06	0.82 ± 0.08	0.83 ± 0.07	0.84 ± 0.10
	relative to body (%)	4.28 ± 0.33	4.49 ± 0.36	4.49 ± 0.36	4.98 ± 0.61** (↑16)
	relative to brain (%)	174.69 ± 14.42	186.61 ± 18.84	186.45 ± 13.52	197.22 ± 21.68
Kidney -	absolute (g)	0.261 ± 0.025	0.257 ± 0.018	0.250 ± 0.016	0.235 ± 0.018* (↓10)
	relative to body (%)	1.410 ± 0.091	1.407 ± 0.091	1.348 ± 0.086	1.394 ± 0.116
	relative to brain (%)	57.551 ± 3.835	58.397 ± 3.053	55.728 ± 3.665	55.266 ± 5.112

a Data were obtained from Tables 5 through 7 on pages 60-69 of the study report; n=9-10. Percent difference from controls, calculated by the reviewers, is included in parentheses.

* Significantly different from the control group at p≤0.05

** Significantly different from the control group at p≤0.01

*** Significantly different from the control group at p≤0.001

2. Gross pathology - There were no treatment-related macroscopic findings.

3. Microscopic pathology - Slight centrilobular hepatocellular hypertrophy was observed in the ≥1000 ppm males (1-9/10 treated vs 0/10 controls) and in the 2000 ppm females (2/10 treated vs 0/10 controls; Table 7). Increased incidences of slight diffuse acinar hypertrophy were observed in the submaxillary salivary glands of the ≥1000 ppm females (4-8/10 treated vs 1/10 controls). There were no other treatment-related microscopic findings.

Table 7. Selected mean (±SD) organ weights in mice treated with Thidiazuron in the diet for 90 days [a]

Parameter	Dose (ppm)			
	0	500	1000	2000
Males				
Liver - slight centrilobular hepatocellular hypertrophy	0	0	1	9 [b]
Females				
Liver - slight centrilobular hepatocellular hypertrophy	0	0	0	2
Submaxillary salivary gland - slight diffuse acinar hypertrophy	1	1	4	8

a Data were obtained from Table 9 (I and II) on pages 77, 84, 85, 90-97 of the study report; n=10 (includes decedents).

b Hypertrophy was found in the liver in 8/9 males examined at the terminal sacrifice and in the one decedent.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - It was concluded that the LOAEL was 1000 ppm based on decreased total cholesterol in the males and increased incidences of slight diffuse acinar hypertrophy in the submaxillary salivary glands in 4/9 females.

B. REVIEWER COMMENTS - All mice in the 4000 ppm group died or were sacrificed moribund on Days 6-9. There were no other treatment-related mortalities. One 2000 ppm male was sacrificed on Day 20 after accidental trauma. One 1000 ppm female was found dead on Day 51; a brain hemorrhage observed at necropsy indicated accidental trauma.

In the 4000 ppm group, animals exhibited the following clinical signs of toxicity prior to their death or sacrifice: (i) reduced motor activity, prostration, piloerection, dyspnea, cold to touch, thin appearance, and hunched posture in both sexes; (ii) staggering step in males; and (iii) no feces in females. Body weights were severely decreased (28-29%; $p \leq 0.001$) in the two males and three females still alive on Day 8. Food consumption was severely decreased (↓53-62%; $p \leq 0.001$) in both sexes for Days 1-8.

At 2000 ppm, hunched posture was observed in two males and one female on Day 15, and reduced motor activity was noted in one female on Days 28-29. Body weights were decreased

($p \leq 0.05$) throughout treatment in the males, except Days 57 and 90 (↓4-7%), and throughout treatment in the females (↓6-17%). Overall (Days 1-90) body weight gains at this dose were decreased in the males (↓19%; not significant) and females (↓40%; $p \leq 0.001$). Weekly food consumption was decreased throughout treatment in the females (↓7-23%), resulting in decreased (↓12%; $p \leq 0.01$) average food consumption for the overall (Days 1-90) study.

At ≥1000 ppm in the males, cholesterol was dose-dependently decreased (↓16-34%; $p \leq 0.05$). Additionally in the 2000 ppm males, alkaline phosphatase was increased (↑14%; $p \leq 0.05$), and albumin was decreased (↓9%; $p \leq 0.05$). At 2000 ppm in both sexes, terminal body weights were decreased (↓5-9%; $p \leq 0.05$), and relative (to body) liver weights were increased (↑10-16%; $p \leq 0.01$). Slight centrilobular hepatocellular hypertrophy was observed in the ≥1000 ppm males (1-9/10 treated vs 0/10 controls) and in the 2000 ppm females (2/10 treated vs 0/10 controls).

In the ≥1000 ppm males, absolute, relative (to body), and relative (to brain) kidney weights were decreased (↓9-15%; $p \leq 0.01$). Absolute kidney weights were decreased in the 2000 ppm females (↓10%; $p \leq 0.05$). The organ weight changes in the kidney were considered equivocal because there were no macroscopic or microscopic findings in the kidney. Increased incidences of slight diffuse acinar hypertrophy were observed in the submaxillary salivary glands of the ≥1000 ppm females (4-8/10 treated vs 1/10 controls).

At 500 ppm, no treatment-related effects were found.

The LOAEL is 1000 ppm (equivalent to 170.9/202.6 mg/kg/day in M/F) based on decreased cholesterol in the males and on increased incidences of centrilobular hepatocellular hypertrophy in the males and diffuse acinar hypertrophy in the salivary glands in the females. The NOAEL is 500 ppm (equivalent to 85.2/99.8 mg/kg/day in M/F).

This study is classified **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.3100; OECD 408) for a 90-day oral toxicity study in the mouse.

C. <u>STUDY DEFICIENCIES</u> - The following deficiencies were noted, but do not change the conclusions of this DER:

- No hematology parameters were evaluated.
- Serum electrolytes, sorbitol dehydrogenase, gamma glutamyl transferase, creatinine, and glucose were not measured.
- Ophthalmoscopic examinations were not performed.
- Epididymides, ovaries, and uterus were not weighed.
- The nose and pharynx were not examined microscopically.

DATA FOR ENTRY INTO ISIS

Subchronic Oral Study - rodent (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Endpoints	Comments
120301	46121505	subchronic	mouse	90 days	oral	diet	85.2-383.9	0/0, 85.2/99.8, 170.9/202.6, 351.4/383.9 [M/F]	85.2	170.9	Clinical signs BW, BWG, FC Clinical chemistry Liver	

DATA EVALUATION RECORD

THIDIAZURON

Study Type: §82-1a, Subchronic Oral Toxicity Study in Rats

Work Assignment No. 1-01-17 F (MRIDs 46121506 and 46121509)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Signature: Steven Brecher
Date: 3/19/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature:** Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 10/26/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 10/28/04
EPA Work Assignment Manager: P. V. Shah, Ph.D. **Signature:** Shazi Dawan
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity [feeding] - rat; OPPTS 870.3100a [§82-1a]; OECD 408.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (99.5% a.i.)

SYNONYMS: 1-Phenyl-3-(1,2,3-thiadiazol-5-yl)urea

CITATION:

Foulon, O. (2001) Thidiazuron: 90-day toxicity study in the rat by dietary administration. Aventis Crop Science, 355, rue Dostoïevski, Sophia Antipolis, France. Laboratory Study SA 00491, October 22, 2001. MRID 46121506. Unpublished.

Steiblen, G. (2003) Thidiazuron: 28-day toxicity study in the rat by dietary administration (investigation of renal function). Bayer Crop Science, 355, rue Dostoïevski, Sophia Antipolis, France. Laboratory Study SA 01245, June 13, 2003. MRID 46121509. Unpublished (This was a special study which was designed to evaluate renal function, and as such a complete DER was not prepared for this study. The findings were summarized in this DER).

SPONSOR: Aventis Crop Science, 14-20, rue Pierre Baizet, Lyon, France.

EXECUTIVE SUMMARY: In a subchronic oral toxicity study (MRID 46121506), thidiazuron (99.5% a.i., Batch # 107623-03) was administered to 10 Wistar (RJ:WI[IOPS HAN]) rats/sex/dose in the diet at dose levels of 0, 200, 600, 1800, 5400, or 16,200 ppm (equivalent to 0/0, 11.2/14.0, 34.5/42.1, 102/123, 294/325, and [highest dose not calculated] mg/kg/day) for 90 days.

No treatment-related effects were observed at **200 or 600 ppm**, or on ophthalmology or hematology at any dose.

At >=**1800 ppm** in the males, decreased body weights, overall (Days 1-90) body weight gains, and food consumption were observed. Increased serum alkaline phosphatase was noted. Decreases were noted in absolute epididymides weight and absolute and relative (to body) prostate gland weight, and small prostate gland and small seminal vesicles were observed. The following alterations in microscopic pathology were noted: (i) slight to mild diminished secretion of the prostate gland; (ii) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland; (iii) slight to mild vacuolation/mineralization glomerulopathy of the kidney; (iv) slight to mild bilateral hyperplasia of the pelvic epithelium of the kidney; (v) slight to mild mineralized concretions of the renal pelvis; (vi) slight focal mononuclear cell inflammation of the kidney; (vii) slight to mild centrilobular hepatocellular hypertrophy; (viii) slight to mild hyperplasia of germinal centers in the spleen; (ix) slight to marked diminished secretion of the seminal vesicle; (x) slight to moderate diffuse atrophy of the mammary gland; and (xi) slight to mild germinal centers in the medulla of the thymus.

At >=**1800 ppm** in the females, the following alterations in microscopic pathology were noted: (i) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland; (ii) slight to mild vacuolation/mineralization glomerulopathy of the kidney; (iii) slight centrilobular hepatocellular hypertrophy; (iv) slight to moderate adipose infiltration of the bone and marrow of the sternum; (v) slight to mild hypertrophy of the interstitial gland of the ovary; and (vi) slight to moderate hyperplasia of germinal centers in the spleen.

At >=**1800 ppm**, increased urea was observed in both sexes.

At **5400 ppm** in the males, the following increases in relative organ weights were observed: (i) adrenal gland; (ii) kidney; (iii) liver; and (iv) spleen. Relative testes weight was decreased. Additionally, the following alterations in microscopic pathology were observed: (i) slight focal mononuclear cell inflammation of the epididymis; (ii) slight focal mineralization of the inner medulla of the kidney; (iii) slight to mild adipose infiltration of the bone and marrow of the sternum; (iv) slight focal foamy alveolar macrophages; (v) slight focal hemorrhage of the lung; (vi) slight to mild germinal centers of the mesenteric and inguinal lymph nodes; and (vii) slight unilateral atrophy of the seminiferous epithelium of the testes.

At **5400 ppm** in the females, decreased body weights, overall (Days 1-90) body weight gains, and food consumption were observed. Increased serum alkaline phosphatase and phosphorus were noted. Decreases were noted in absolute and relative adrenal gland and uterus weights. Increases in relative kidney and liver weights were observed, and decreased absolute mesenteric lymph node weight was noted. Small uterus was observed. The following alterations in microscopic pathology were observed: (i) slight to mild atrophy of the zona reticularis of the adrenal gland; (ii) slight brown pigment in the zona reticularis of the adrenal gland; (iii) no cyclical activity of the uterus; (iv) slight to marked diffuse atrophy of the uterus; (v) slight to marked diffuse atrophy of the vagina; (vi) reduced numbers of recent corpora lutea in the ovary; (vii) slight to mild diffuse atrophy of the mammary gland; (viii) slight germinal centers of the mesenteric and inguinal lymph nodes; and (ix) slight to mild atrophy of the trabecular bone.

At **5400 ppm** in both sexes, rats exhibited piloerection, appeared thin, and excreted few feces. Increased cholesterol and potassium were observed, and urine volume was increased.
At **16,200 ppm**, males were observed to have hunched posture, and all animals demonstrated deficits in righting, grasping, corneal, pupillary, head shaking, and auditory startle reflexes. All animals in this dose group were either found dead or were sacrificed moribund by Day 10.

The LOAEL is 1800 ppm (equivalent to 102/123 mg/kg/day [M/F]), based on decreased body weights, overall (Days 1-90) body weight gains, and food consumption, increased serum alkaline phosphatase, decreased absolute epididymides and absolute and relative (to body) prostate gland weight, small prostate and small seminal vesicles, and microscopic findings in the prostate gland, seminal vesicle, mammary gland, and thymus of the males, microscopic findings in the bone and marrow of the sternum and ovary of the females, and increased urea and microscopic findings in the adrenal gland, kidney, liver, and spleen of both sexes. The NOAEL is 600 ppm (equivalent to 34.5/42.1 mg/kg/day [M/F]).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

EXECUTIVE SUMMARY for 28-day toxicity study in the rat (MRID 46121509) :

After the conclusion of the subchronic (90-day) toxicity study, an abbreviated 28-day oral toxicity study (MRID 46121509) was performed specifically to investigate renal function and to provide a dose rationale for future studies in rats treated with thidiazuron.

In this subchronic oral toxicity study (MRID 46121509), thidiazuron (99.5% a.i., Batch # 107623-03) was administered to 10 male Wistar (RJ:WI[IOPS HAN]) rats/dose in the diet at dose levels of 0, 900, 1800, or 3600 ppm (equivalent to 0, 67, 135, and 254 mg/kg/day) for 28 days. These doses were in between doses of the 90-day study (0, 200, 600, 1800, 5400, or 16,200 ppm (equivalent to 0/0, 11.2/14.0, 34.5/42.1, 102/123, 294/325, and [highest dose not calculated] mg/kg/day). Detailed DER will not be prepared for this study. A summary of this supplementary study is given below.

No effects of treatment were observed on mortality, clinical signs of toxicity, or water consumption.

At ≥1800 ppm, decreased ($p \leq 0.01$) serum glucose was observed (↓13-22%). Urinary pH was decreased (pH 6.5-6.6; $p \leq 0.05$) compared to controls (pH 7.1). At necropsy, small seminal vesicles (3-9/10 treated vs 0/10 controls), small prostate gland (2-8/10 treated vs 0/10 controls),

and prominent liver lobulation (2-4/10 treated vs 0/10 controls) were observed. Microscopic pathology revealed focal/multifocal basophilic tubules (6-7/10 treated vs 0/10 controls) in the kidneys.

At 3600 ppm, decreased ($p \leq 0.01$) body weights were observed on Days 4-28 (↓12-20%), decreased ($p \leq 0.01$) body weight gains were noted on Days 4-15 (↓31-125%), and decreased ($p \leq 0.05$) food consumption was observed on Days 4-28 (↓15-44%). Increased ($p \leq 0.01$) serum cholesterol (↑29%) was noted. Urinary potassium was decreased (↓33%; $p \leq 0.05$), and relative (to body) kidney weight was increased (↑12%; $p \leq 0.01$). Also in the kidney, microscopic pathology revealed multifocal diffuse glomerulopathy with vacuolation (8/10 treated vs 0/10 controls) and unilateral pelvic dilatation (4/10 treated vs 1/10 control).

The LOAEL is 1800 ppm (equivalent to 135 mg/kg/day), based on decreased serum glucose and urinary pH, small seminal vesicles and prostate gland, prominent liver lobulation, and focal/multifocal basophilic tubules in the kidneys. The NOAEL is 900 ppm (equivalent to 67 mg/kg/day).

This study is classified as **acceptable for 28-day special study/non-guideline.**

The following table summarizes NOAEL/LOAELs of the 28- and 90-day oral toxicity studies.

	28-day oral toxicity study	90-day oral toxicity study
NOAEL (mg/kg/day)	67	34.5
LOAEL (mg/kg/day)	135	102

(table continues next page)

prostate gland weight, and small prostate gland and small seminal vesicles were observed. The following alterations in microscopic pathology were noted: (i) slight to mild diminished secretion of the prostate gland; (ii) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland; (iii) slight to mild vacuolation/mineralization glomerulopathy of the kidney; (iv) slight to mild bilateral hyperplasia of the pelvic epithelium of the kidney; (v) slight to mild mineralized concretions of the renal pelvis; (vi) slight focal mononuclear cell inflammation of the kidney; (vii) slight to mild centrilobular hepatocellular hypertrophy; (viii) slight to mild hyperplasia of germinal centers in the spleen; (ix) slight to marked diminished secretion of the seminal vesicle; (x) slight to moderate diffuse atrophy of the mammary gland; and (xi) slight to mild germinal centers in the medulla of the thymus.

At **>=1800 ppm** in the females, the following alterations in microscopic pathology were noted: (i) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland; (ii) slight to mild vacuolation/mineralization glomerulopathy of the kidney; (iii) slight centrilobular hepatocellular hypertrophy; (iv) slight to moderate adipose infiltration of the bone and marrow of the sternum; (v) slight to mild hypertrophy of the interstitial gland of the ovary; and (vi) slight to moderate hyperplasia of germinal centers in the spleen.

At **>=1800 ppm**, increased urea was observed in both sexes.

At **5400 ppm** in the males, the following increases in relative organ weights were observed: (i) adrenal gland; (ii) kidney; (iii) liver; and (iv) spleen. Relative testes weight was decreased. Additionally, the following alterations in microscopic pathology were observed: (i) slight focal mononuclear cell inflammation of the epididymis; (ii) slight focal mineralization of the inner medulla of the kidney; (iii) slight to mild adipose infiltration of the bone and marrow of the sternum; (iv) slight focal foamy alveolar macrophages; (v) slight focal hemorrhage of the lung; (vi) slight to mild germinal centers of the mesenteric and inguinal lymph nodes; and (vii) slight unilateral atrophy of the seminiferous epithelium of the testes.

At **5400 ppm** in the females, decreased body weights, overall (Days 1-90) body weight gains, and food consumption were observed. Increased serum alkaline phosphatase and phosphorus were noted. Decreases were noted in absolute and relative adrenal gland and uterus weights. Increases in relative kidney and liver weights were observed, and decreased absolute mesenteric lymph node weight was noted. Small uterus was observed. The following alterations in microscopic pathology were observed: (i) slight to mild atrophy of the zona reticularis of the adrenal gland; (ii) slight brown pigment in the zona reticularis of the adrenal gland; (iii) no cyclical activity of the uterus; (iv) slight to marked diffuse atrophy of the uterus; (v) slight to marked diffuse atrophy of the vagina; (vi) reduced numbers of recent corpora lutea in the ovary; (vii) slight to mild diffuse atrophy of the mammary gland; (viii) slight germinal centers of the mesenteric and inguinal lymph nodes; and (ix) slight to mild atrophy of the trabecular bone.

At **5400 ppm** in both sexes, rats exhibited piloerection, appeared thin, and excreted few feces. Increased cholesterol and potassium were observed, and urine volume was increased.

At **16,200 ppm**, males were observed to have hunched posture, and all animals demonstrated deficits in righting, grasping, corneal, pupillary, head shaking, and auditory startle reflexes. All animals in this dose group were either found dead or were sacrificed moribund by Day 10.

The LOAEL is 1800 ppm (equivalent to 102/123 mg/kg/day [M/F]), based on decreased body weights, overall (Days 1-90) body weight gains, and food consumption, increased serum alkaline phosphatase, decreased absolute epididymides and absolute and relative (to body) prostate gland weight, small prostate and small seminal vesicles, and microscopic findings in the prostate gland, seminal vesicle, mammary gland, and thymus of the males, microscopic findings in the bone and marrow of the sternum and ovary of the females, and increased urea and microscopic findings in the adrenal gland, kidney, liver, and spleen of both sexes. The NOAEL is 600 ppm (equivalent to 34.5/42.1 mg/kg/day [M/F]).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

EXECUTIVE SUMMARY for 28-day toxicity study in the rat (MRID 46121509) **:**

After the conclusion of the subchronic (90-day) toxicity study, an abbreviated 28-day oral toxicity study (MRID 46121509) was performed specifically to investigate renal function and to provide a dose rationale for future studies in rats treated with thidiazuron.

In this subchronic oral toxicity study (MRID 46121509), thidiazuron (99.5% a.i., Batch # 107623-03) was administered to 10 male Wistar (RJ:WI[IOPS HAN]) rats/dose in the diet at dose levels of 0, 900, 1800, or 3600 ppm (equivalent to 0, 67, 135, and 254 mg/kg/day) for 28 days. These doses were in between doses of the 90-day study (0, 200, 600, 1800, 5400, or 16,200 ppm (equivalent to 0/0, 11.2/14.0, 34.5/42.1, 102/123, 294/325, and [highest dose not calculated] mg/kg/day). Detailed DER will not be prepared for this study. A summary of this supplementary study is given below.

No effects of treatment were observed on mortality, clinical signs of toxicity, or water consumption.

At ≥1800 ppm, decreased ($p \leq 0.01$) serum glucose was observed (↓13-22%). Urinary pH was decreased (pH 6.5-6.6; $p \leq 0.05$) compared to controls (pH 7.1). At necropsy, small seminal vesicles (3-9/10 treated vs 0/10 controls), small prostate gland (2-8/10 treated vs 0/10 controls), and prominent liver lobulation (2-4/10 treated vs 0/10 controls) were observed. Microscopic pathology revealed focal/multifocal basophilic tubules (6-7/10 treated vs 0/10 controls) in the kidneys.

At 3600 ppm, decreased ($p \leq 0.01$) body weights were observed on Days 4-28 (↓12-20%), decreased ($p \leq 0.01$) body weight gains were noted on Days 4-15 (↓31-125%), and decreased ($p \leq 0.05$) food consumption was observed on Days 4-28 (↓15-44%). Increased ($p \leq 0.01$) serum cholesterol (↑29%) was noted. Urinary potassium was decreased (↓33%; $p \leq 0.05$), and relative (to body) kidney weight was increased (↑12%; $p \leq 0.01$). Also in the kidney, microscopic pathology revealed multifocal diffuse glomerulopathy with vacuolation (8/10 treated vs 0/10 controls) and unilateral pelvic dilatation (4/10 treated vs 1/10 control).

The LOAEL is 1800 ppm (equivalent to 135 mg/kg/day), based on decreased serum glucose and urinary pH, small seminal vesicles and prostate gland, prominent liver lobulation, and focal/multifocal basophilic tubules in the kidneys. The NOAEL is 900 ppm (equivalent to 67 mg/kg/day).

This study is classified as **acceptable for 28-day special study/non-guideline.**

The following table summarizes NOAEL/LOAELs of the 28- and 90-day oral toxicity studies.

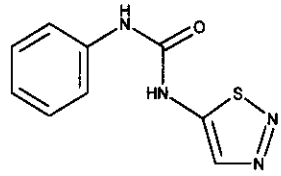
	28-day oral toxicity study	90-day oral toxicity study
NOAEL (mg/kg/day)	67	34.5
LOAEL (mg/kg/day)	135	102

(table continues next page)

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Thidiazuron

Description:	Light yellow powder
Batch #:	107623-03
Purity:	99.5% a.i.
Compound Stability:	Stable in the diet for at least 6 weeks at room temperature
CAS # of TGAI:	51707-55-2
Structure:	

2. Vehicle: Diet

3. Test animals:

Species:	Rat	
Strain:	Wistar (RJ:WI[IOPS HAN])	
Age/weight at study initiation:	7 weeks old; 240-273 g males, 161-187 g females	
Source:	R. Janvier, Le Genest St Isle, France	
Housing:	Individually in suspended stainless steel wire mesh cages	
Diet:	"M 20 contrôlé" certified rodent powder diet (Pietrement, Provins, France), *ad libitum*	
Water:	Filtered and softened tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	40-70%
	Air changes:	10-15/hr
	Photoperiod:	12 hrs light/12 hrs dark
Acclimation period:	7 days	

B. STUDY DESIGN:

1. In life dates: Start: October 25, 2000 End: January 26, 2001

2. Animal assignment: Animals were randomly assigned (stratified by weight) to the test groups presented in Table 1.

Table 1: Study design[a]

Test Group	Nominal Dose (ppm)	Mean Chemical Intake (mg/kg bw/day) [M/F]	# of Males	# of Females
Control	0	0/0	10	10
Low	200	11.2/14.0	10	10
Mid 1	600	34.5/42.1	10	10
Mid 2	1800	102/123	10	10
Mid 3	5400	294/325	10	10
High	16,200	Not calculated[b]	10	10

a Data were obtained from pages 16 and 24 of MRID 46121506.
b All animals in this group died or were sacrificed in moribund condition by Day 10.

3. Dose selection rationale: No dose selection rationale was provided. The concurrently submitted 28-day oral toxicity study (MRID 46121509) was initiated 2 days prior to the completion of this 90-day study.

4. Treatment preparation, administration, and analysis: Dietary formulations were prepared by mixing a weighed amount of the test substance with an appropriate amount of feed to yield the desired concentration. Dietary formulations were prepared approximately every 3 weeks and stored at approximately -15°C until use. Homogeneity (top, middle, and bottom) was tested in the 200 and 16,200 ppm dietary formulations from a trial preparation and the first preparation. Concentration analyses were performed on all dose levels for each preparation. Stability analyses were performed on the first preparation at 200 and 16,200 ppm. Stability was determined following storage for up to 6 weeks at room temperature.

Results

Homogeneity Analysis: 200 ppm = 93-113% of nominal
16,200 ppm = 90-113% of nominal

Stability Analysis: % of time 0: 97-108%

Concentration Analysis:

Dose (ppm)	Range (% of nominal)
200	94-102
600	88-98
1800	93-102
5400	87-106
16,200	103-108

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics: Endpoints analyzed included body weights, body weight gains, food consumption, compound intake, hematology, clinical chemistry, urinalysis, and organ weights. Levene's Test was used to determine homogeneity of variance ($p \leq 0.05$). If Levene's test did not reject the hypothesis of homogeneous variances, treatment groups were compared using parametric ANOVA. If significance was found, Dunnett's test was used to compare treatment groups to controls. If Levene's test indicated lack of homogeneity of variance, robust linear regression methods were used. Overall treatment group differences were compared with Wald chi-square tests, followed by individual t-tests for exposed vs control group comparisons when the overall treatment effect was significant. The level denoting significance was $p \leq 0.05$, $p \leq 0.01$, or $p \leq 0.001$ for all analyses.

C. METHODS:

1. Observations:

a. Cageside Observations: Animals were inspected for moribundity and mortality twice daily (once daily on weekends and holidays). Clinical signs of toxicity were recorded at least once daily.

b. Clinical Examinations: Detailed clinical examinations were conducted during acclimation and at least weekly during treatment.

c. Neurological Evaluations: The following reflexes were measured during acclimation and on Week 12: grasping, righting, corneal, pupillary, auditory startle, and head shaking.

2. Body weight: Animals were weighed during acclimation, on the first day of treatment, generally at weekly intervals throughout treatment, and just prior to necropsy.

3. Food consumption and compound intake: Mean food consumption (g/animal/day) was generally recorded for each animal weekly. Food efficiency was not calculated. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight data.

4. Ophthalmoscopic examination: Ophthalmoscopic examinations were performed on all animals during the acclimation period, and on all surviving animals in the control and 5400 ppm groups at Week 12.

5. Hematology & Clinical Chemistry: Blood samples were collected from all surviving animals for hematology at Weeks 4 and 9, and for hematology and clinical chemistry just prior to sacrifice. Blood was collected from the retro-orbital plexus of fasted (overnight) anesthetized animals. The CHECKED (X) parameters were examined.

a. Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB concentration (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)	X	Albumin/globulin ratio

X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. Urinalysis: Urine was collected overnight from all surviving animals (food and water fasted) at Week 12. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
X	Volume*	X	Ketones
X	Refractive index	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein*	X	Urobilinogen

* Recommended for 90-day oral rodent studies

7. Sacrifice and Pathology

All animals that died or were sacrificed *in extremis* and those sacrificed on schedule were subjected to gross pathological examination. Animals were fasted overnight prior to sacrifice. The CHECKED (X) tissues were collected for histological examination and the (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	XX	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**	X	Harderian/lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroid*
X	Rectum*	X	Urinary bladder*	XX	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum)
	Bile duct (rat)	XX	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Femorotibial articular surface
X	Lung*	X	Mammary gland*		
	Nose*	XX	Cervix		
	Pharynx*	X	Vagina		
X	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies

Samples were fixed in neutral buffered 10% formalin, with the exception of the eye, optic nerve, Harderian gland, epididymis, and testis, which were fixed in Davidson's fixative. Histopathological examinations were performed on all tissues from all animals in the control and 5400 ppm groups, and on all decedents in the intermediate dose groups. Additionally, adrenal gland, bone, bone marrow, femorotibial articular surface, kidney, liver, lung, mammary gland, mesenteric lymph node, ovary, prostate gland, seminal vesicle, spleen, submaxillary lymph node, thymus, uterus, and vagina were examined microscopically in the intermediate dose groups. The findings of the consultant pathologist were peer reviewed by the study pathologist, and all findings represent the consensus opinion of the two pathologists. Additionally, a 28-day toxicity study investigating renal function was performed after completion of the 90-day study and is included as an Appendix to this DER.

II. RESULTS

A. OBSERVATIONS:

1. Clinical signs of toxicity: All ≥5400 ppm rats exhibited piloerection (first observed on Day 6), appeared thin (first observed on Day 4), and excreted few feces (first observed on Day 6). In addition, the 16,200 ppm males had a hunched posture (70% treated vs 0% controls; first observed on Day 8). No other treatment-related clinical signs of toxicity were observed.

2. Mortality: At 16,200 ppm, all animals were either found dead or were sacrificed moribund by Day 10; therefore, this group will not be discussed beyond Observations. One 1800 ppm male died during anesthesia prior to blood sampling; this death was not considered treatment-related. No other deaths were observed.

3. Neurological Evaluations: At 16,200 ppm, all animals demonstrated deficits in all reflexes tested (righting, grasping, corneal, pupillary, head shaking, and auditory startle). All other groups demonstrated normal reflexes.

B. BODY WEIGHT AND WEIGHT GAIN: Body weights and body weight gains are presented in Tables 2a and b, respectively. Decreased ($p \leq 0.01$) body weights were observed throughout treatment in the ≥1800 ppm males (↓7-33%) and in the 5400 ppm females (↓15-26%). Overall (Days 1-90) body weight gains were also decreased ($p \leq 0.01$) in the ≥1800 ppm males (↓27-52%) and in the 5400 ppm females (↓43%). Additionally in the 1800 ppm females, body weights were decreased ($p \leq 0.05$) on Days 9-22, 36, and 50 (↓5-8%), and body weight gains were transiently decreased from Days 1-15 (↓31%; $p \leq 0.05$). These findings were minor, transient, and not considered adverse. No treatment-related effects were observed on body weights or body weight gains at 200 or 600 ppm.

Table 2a. Mean (± SD) body weights (g) in rats treated with thidiazuron in the diet for at least 90 days.[a]

Study day	Dose (ppm)				
	0	200	600	1800	5400
Males					
Day 1	257.8±6.9	259.6±7.5	256.6±7.4	257.2±8.0	259.3±5.8
Day 9	325.9±12.0	324.8±16.1	316.6±22.6	303.6±12.3*** (↓7)	231.6±8.8*** (↓29)
Day 22	382.4±18.1	386.1±24.5	369.7±38.0	338.2±25.0** (↓12)	257.6±15.2*** (↓33)
Day 90	520.0±40.5	523.5±40.9	512.5±46.7	448.6±38.5** (↓14)	386.2±46.0*** (↓26)
Females					
Day 1	177.3±6.5	177.6±5.7	176.1±6.5	175.3±5.3	175.5±6.3
Day 9	203.5±7.6	204.6±9.2	203.0±9.2	193.2±7.0* (↓5)	155.2±5.7***(↓24)
Day 15	213.4±10.8	215.0±12.4	212.4±13.3	200.3±6.7* (↓6)	158.2±9.8*** (↓26)
Day 22	228.0±14.4	227.9±14.7	224.5±15.2	210.2±7.4* (↓8)	170.8±11.1*** (↓25)
Day 90	271.4±17.2	283.2±14.3	279.5±15.1	262.3±14.7	229.6±13.8*** (↓15)

a Data were obtained from pages 53-55 and 57-59 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; p≤0.05

** Significantly different from controls; p≤0.01

*** Significantly different from controls; p≤0.001

Table 2b. Mean (± SD) body weight gains (g) in rats treated with thidiazuron in the diet for at least 90 days.[a]

Interval	Dose (ppm				
	0	200	600	1800	5400
Males					
Day 1-15	91.6±12.2	92.9±12.8	81.9±23.8	60.3±17.8*** (↓34)	-17.8±12.4*** (↓119)
Day 1-29	138.1±22.4	139.4±21.0	125.4±33.8	91.2±27.9** (↓34)	13.7±20.8*** (↓90)
Day 1-56	222.9±32.1	221.4±31.8	207.6±35.8	148.1±36.3*** (↓34)	77.3±38.4*** (↓65)
Day 1-90	262.2±40.0	263.9±36.4	255.9±42.9	190.7±39.7** (↓27)	126.9±47.2*** (↓52)
Females					
Day 1-15	36.1±10.1	37.4±7.4	36.3±9.9	25.0±5.6* (↓31)	-17.3±9.6*** (↓148)
Day 1-29	53.7±13.0	57.4±7.8	54.3±10.3	43.6±5.8	4.6±10.6*** (↓91)
Day 1-56	83.3±16.5	91.8±14.1	88.8±9.5	71.1±10.4	35.0±9.7*** (↓58)
Day 1-90	94.1±16.9	105.6±10.2	103.4±11.9	87.0±13.5	54.1±14.4*** (↓43)

a Data were obtained from pages 55-56 and 59-60 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; p≤0.05

** Significantly different from controls; p≤0.01

*** Significantly different from controls; p≤0.001

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption: Decreased ($p \leq 0.05$) food consumption was generally observed throughout the study (↓11-66%; Table 3) and overall (Days 1-90; ↓12-32%; $p \leq 0.01$) in the ≥1800 ppm males. Food consumption was also decreased ($p \leq 0.01$) in the 5400 ppm females (↓16-69%). Additionally in the 1800 ppm females, food consumption was decreased ($p \leq 0.001$) on Days 1-36 (↓11-20%) and overall (Days 1-90; ↓9%; $p \leq 0.05$); however, this effect was minor and no corresponding decreases were observed on body weights or body weight gains. Therefore, this effect was not considered adverse. No treatment-related effect was observed on food consumption at 200 or 600 ppm..

Table 3. Mean (± SD) food consumption (g/animal/day) in rats treated with thidiazuron in the diet for at least 90 days.[a]

Interval	Dose (ppm)				
	0	200	600	1800	5400
Males					
Day 1-9	27.4±1.8	27.3±2.1	26.3±2.8	22.6±1.8*** (↓18)	9.3±2.1*** (↓66)
Day 78-84	23.9±2.4	23.8±2.3	25.0±2.6	21.5±2.5 (↓10)	19.6±3.2** (↓18)
Day 84-90	22.5±1.8	21.8±1.4	22.9±1.5	20.1±2.0* (↓11)	18.2±2.5*** (↓19)
Overall (Day 1-90)	24.4±1.2	24.5±1.7	24.2±1.6	21.4±1.8** (↓12)	16.6±1.8*** (↓32)
Females					
Day 1-9	17.9±1.2	17.9±1.3	17.7±1.8	14.3±1.2*** (↓20)	5.6±1.3*** (↓69)
Day 29-36	18.6±1.6	18.3±0.9	18.3±2.0	16.5±1.2*** (↓11)	15.0±3.4**(↓19)
Day 36-43	17.9±1.2	18.3±0.8	18.0±1.3	16.9±1.1	15.1±1.9*** (↓16)
Day 84-90	16.3±1.4	16.7±2.0	15.7±1.2	15.3±1.7	13.0±1.9*** (↓20)
Overall (Day 1-90)	17.3±1.3	17.5±0.9	17.2±1.3	15.8±1.0* (↓9)	11.9±0.6*** (↓31)

a Data were obtained from pages 62-64 and 67-69 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.
* Significantly different from controls; $p \leq 0.05$
** Significantly different from controls; $p \leq 0.01$
*** Significantly different from controls; $p \leq 0.001$

2. Compound consumption: The mean achieved dosages (mg/kg bw/day) based on actual body weights, food consumption, and nominal dosages are presented in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No treatment-related effects were observed during ophthalmological examination.

E. BLOOD ANALYSES

1. Hematology: No treatment-related hematology findings were observed. At terminal sacrifice in the 5400 ppm females, decreased ($p \leq 0.05$) platelets were observed (↓14%), but this finding

was not corroborated by gross or microscopic pathology. Several other differences ($p \leq 0.05$) from controls were observed in the treated groups; however, these findings were minor, transient, and/or not clearly dose-dependent.

2. Clinical Chemistry: Clinical chemistry findings are presented in Table 4. Increased ($p \leq 0.05$) urea was observed in the 1800 ppm group males and females (↑19-23%). Increased ($p \leq 0.01$) alkaline phosphatase was noted in the ≥1800 ppm males (↑31-39%) and 5400 ppm females (↑102%). In the 5400 ppm group males and females, increased ($p \leq 0.01$) cholesterol (↑42-51%) and potassium (↑9-16%) were observed. In the 5400 ppm females, increased ($p \leq 0.05$) phosphorus was noted (↑18%). Increased ($p \leq 0.05$) urea was observed in the 600 ppm males (↑17%); however, there were no other corroborating findings at this dose level. Therefore, this finding is considered equivocal. All other differences ($p \leq 0.05$) were minor, incidental, and were not considered adverse.

Table 4. Mean (± SD) clinical chemistry findings in rats treated with thidiazuron in the diet for at least 90 days.[a]

Parameter	Dose (ppm)				
	0	200	600	1800	5400
Males (n=9-10)					
Urea (mmol/L)	5.009±0.670	5.198±0.799	5.880±0.632* (↑17)	6.153±0.614** (↑23)	6.137±0.579** (↑23)
Alkaline Phosphatase (IU/L)	54.7±5.5	54.6±10.3	59.7±13.7	71.9±13.9*** (↑31)	76.0±22.0** (↑39)
Cholesterol (mmol/L)	1.742±0.214	1.826±0.281	2.080±0.417	2.107±0.314	2.479±0.329*** (↑42)
Potassium (mmol/L)	3.59±0.14	3.67±0.18	3.67±0.16	3.74±0.24	3.92±0.22** (↑9)
Females (n=9-10)					
Urea (mmol/L)	5.385±0.744	5.579±0.927	5.588±0.501	6.395±1.010* (↑19)	6.550±0.686* (↑22)
Alkaline Phosphatase (IU/L)	38.7±10.3	36.3±5.9	40.0±11.0	39.9±10.0	78.2±50.0** (↑102)
Cholesterol (mmol/L)	2.036±0.339	2.267±0.576	1.918±0.305	1.996±0.233	3.077±0.449*** (↑51)
Potassium (mmol/L)	3.33±0.38	3.39±0.29	3.41±0.23	3.40±0.31	3.87±0.30** (↑16)
Phosphorus (mmol/L)	1.316±0.136	1.373±0.156	1.384±0.248	1.345±0.126	1.548±0.149* (↑18)

a Data were obtained from pages 90-97 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; $p \leq 0.05$

** Significantly different from controls; $p \leq 0.01$

*** Significantly different from controls; $p \leq 0.001$

F. Urinalysis: At 5400 ppm, volume was increased ($p \leq 0.01$) in both sexes (↑89-134%; Table 5). No other treatment-related effects on urinalysis parameters were observed.

Table 5. Mean (± SD) urinalysis findings in rats treated with thidiazuron in the diet for at least 90 days.[a]

Parameter	Dose (ppm)				
	0	200	600	1800	5400
Males (n=9-10)					
Volume (mL)	3.50±1.16	3.28±1.99	4.10±1.31	3.98±1.83	6.63±2.62** (↑89)
Females (n=8-10)					
Volume (mL)	2.11±1.04	2.61±1.89	2.57±1.24	2.38±1.20	4.94±2.74** (↑134)

a Data were obtained from pages 99-100 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.

** Significantly different from controls; $p \leq 0.01$

G. SACRIFICE AND PATHOLOGY

1. **Organ weight:** Selected absolute and relative (to body) organ weights are presented in Table 6. Decreased ($p \leq 0.01$) terminal body weights were observed in the ≥1800 ppm males (↓14-26%), and in the 5400 ppm females (↓15%). In the ≥1800 ppm males, decreases ($p \leq 0.05$) were noted in absolute epididymides weight (↓14-26%) and absolute and relative (to body) prostate gland weight (↓38-56%). Additionally in the 5400 ppm males, the following increases ($p \leq 0.01$) in relative organ weights were observed: (i) adrenal gland (↑25%); (ii) kidney (↑23%); (iii) liver (↑28%); and (iv) spleen (↑49%). Relative testes weight was decreased 33% ($p \leq 0.001$). In the 5400 ppm females, decreases ($p \leq 0.05$ or $p \leq 0.001$) were noted in absolute and relative adrenal gland (↓22-34%) and uterus (↓60-67%) weights. Increased ($p \leq 0.001$) relative kidney (↑22%) and liver (↑31%) weights were observed, and decreased ($p \leq 0.05$) absolute mesenteric lymph node weight (↓29%) was noted. Other differences were observed, but were minor, not dose-dependent, and/or an adverse effect was not corroborated by other clinical or pathological data.

Table 6. Selected mean (± SD) absolute and relative (to body) organ weights in rats treated with thidiazuron in the diet for at least 90 days.[a]

Organ		Dose (ppm)				
		0	200	600	1800	5400
Males (n=8-10)						
Terminal body weight (g)		490.5±39.8	494.7±37.8	479.9±41.4	422.4±36.5** (↓14)	361.8±42.7*** (↓26)
Epididymides	absolute (g)	1.459±0.171	1.449±0.123	1.468±0.110	1.260±0.221* (↓14)	1.082±0.197*** (↓26)
Prostate gland	absolute (g)	0.609±0.112	0.602±0.146	0.519±0.168	0.324±0.129*** (↓47)	0.269±0.117*** (↓56)
	relative (%)	0.125±0.025	0.122±0.029	0.108±0.033	0.078±0.032** (↓38)	0.074±0.030** (↓41)
Adrenal gland	relative (%)	0.012±0.002	0.012±0.002	0.012±0.002	0.013±0.002	0.015±0.002** (↑25)
Kidney	relative (%)	0.555±0.044	0.556±0.045	0.564±0.038	0.596±0.032	0.684±0.040*** (↑23)
Liver	relative (%)	2.18±0.17	2.25±0.13	2.44±0.25* (↑12)	2.39±0.17	2.80±0.15*** (↑28)
Spleen	relative (%)	0.187±0.029	0.195±0.027	0.195±0.026	0.198±0.024	0.278±0.075*** (↑49)
Testes	relative (%)	0.758±0.037	0.810±0.172	0.795±0.116	0.884±0.068	1.005±0.156*** (↓33)

Females (n=9-10)						
Terminal body weight (g)		253.0±15.4	265.7±13.5	262.3±14.9	244.9±13.3	214.2±15.0*** (↓15)
Adrenal gland	absolute (g)	0.070±0.020	0.066±0.007	0.066±0.010	0.057±0.011	0.046±0.007*** (↓34)
	relative (%)	0.027±0.007	0.025±0.002	0.025±0.003	0.023±0.004	0.021±0.003* (↓22)
Uterus	absolute (g)	0.590±0.203	0.522±0.093	0.583±0.221	0.498±0.173	0.196±0.093*** (↓67)
	relative (%)	0.232±0.070	0.197±0.036	0.221±0.076	0.203±0.070	0.092±0.045*** (↓60)
Kidney	relative (%)	0.659±0.075	0.653±0.078	0.630±0.053	0.680±0.047	0.801±0.101*** (↑22)
Liver	relative (%)	2.40±0.16	2.40±.0.14	2.40±0.17	2.43±0.23	3.14±0.75*** (↑31)
Mesenteric lymph node	absolute (g)	0.208±0.048	0.200±0.045	0.192±0.051	0.186±0.042	0.148±0.032* (↓29)

a Data were obtained from pages 108-119 in MRID 46121506. Percent differences from controls, calculated by reviewers, are included in parentheses.

* Significantly different from controls; p≤0.05

** Significantly different from controls; p≤0.01

*** Significantly different from controls; p≤0.001

2. Gross pathology: Gross pathology findings are presented in Table 7. In the ≥1800 ppm males, small prostate was observed (3/9-8/10 treated vs 0/10 controls) and small seminal vesicles were noted (4/9-9/10 treated vs 0/10 controls). In the females, small uterus was observed at 5400 ppm (6/10 treated vs 0/10 controls). No other treatment-related findings were noted at necropsy.

Table 7. Gross pathology findings (No. of animals affected/no. of animals examined) in rats treated with thidiazuron in the diet for at least 90 days.[a]

Observation	Dose (ppm)				
	0	200	600	1800	5400
Males (n=9-10)					
Small prostate	0/10	0/10	0/10	3/9	8/10
Small seminal vesicles	0/10	0/10	0/10	4/9	9/10
Females (n=10)					
Small uterus	0/10	0/10	0/10	0/10	6/10

a Data were obtained from pages 130-131 in MRID 46121506.

3. Microscopic pathology: Microscopic pathology findings are presented in Tables 8a and b. In the 600 ppm females, slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland was observed (40% treated vs 0% controls); however, this finding was unsupported by other corroborating pathology data. Therefore, it is considered unrelated to treatment.

In the ≥1800 ppm males, the following were observed: (i) slight to mild diminished secretion of the prostate gland (20-30% treated vs 0% controls); (ii) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland (60-100% treated vs 0% controls); (iii) slight to mild vacuolation/mineralization glomerulopathy of the kidney (70-100% treated vs 0% controls); (iv) slight to mild bilateral hyperplasia of the pelvic epithelium (30-40% treated vs 0% controls); (v)

slight to mild mineralized concretions of the renal pelvis (20-30% treated vs 0% controls); (vi) slight focal mononuclear cell inflammation of the kidney (30-70% treated vs 0% controls); (vii) slight to mild centrilobular hepatocellular hypertrophy (30-90% treated vs 0% controls); (viii) slight to mild hyperplasia of germinal centers in the spleen (50-100% treated vs 0% controls); (ix) slight to marked diminished secretion of the seminal vesicle (40-70% treated vs 0% controls); (x) slight to moderate diffuse atrophy of the mammary gland (20-90% treated vs 0% controls); and (xi) slight to mild germinal centers in the medulla of the thymus (20-50% treated vs 0% controls).

In the ≥1800 ppm females, the following were noted: (i) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland (60-100% treated vs 0% controls); (ii) slight to mild vacuolation/mineralization glomerulopathy of the kidney (50-100% treated vs 0% controls); (iii) slight centrilobular hepatocellular hypertrophy (20-80% treated vs 0% controls); (iv) slight to moderate adipose infiltration of the bone and marrow of the sternum (90-100% treated vs 60% controls); (v) slight to mild hypertrophy of the interstitial gland of the ovary (20-100% treated vs 0% controls); and (vi) slight to moderate hyperplasia of germinal centers in the spleen (50-100% treated vs 0% controls).

In the 5400 ppm males, the following were observed: (i) slight focal mononuclear cell inflammation of the epididymis (80% treated vs 20% controls); (ii) slight focal mineralization of the inner medulla of the kidney (30% treated vs 0% controls); (iii) slight to mild adipose infiltration of the bone and marrow of the sternum (90% treated vs 10% controls); (iv) slight focal foamy alveolar macrophages (40% treated vs 10% controls); (v) slight focal hemorrhage of the lung (60% treated vs 30% controls); (vi) slight to mild germinal centers of the mesenteric (80% treated vs 0% controls) and inguinal (60% treated vs 0% controls) lymph nodes; and (vii) slight unilateral atrophy of the seminiferous epithelium of the testes (50% treated vs 0% controls).

In the 5400 ppm females, the following were noted: (i) slight to mild atrophy of the zona reticularis of the adrenal gland (40% treated vs 0% controls); (ii) slight brown pigment in the zona reticularis of the adrenal gland (40% treated vs 0% controls); (iii) no cyclical activity of the uterus (60% treated vs 0% controls); (iv) slight to marked diffuse atrophy of the uterus (80% treated vs 0% controls); (v) slight to marked diffuse atrophy of the vagina (60% treated vs 0% controls); (vi) reduced numbers of recent corpora lutea in the ovary (20% treated vs 70% controls); (vii) slight to mild diffuse atrophy of the mammary gland (90% treated vs 0% controls); (viii) slight germinal centers of the mesenteric (80% treated vs 0% controls) and inguinal (60% treated vs 0% controls) lymph nodes; and (ix) slight to mild atrophy of the trabecular bone (50% treated vs 0% controls). All other microscopic findings were unaffected by treatment.

(table continues next page)

Table 8a. Microscopic pathology findings (% incidence) in male rats treated with thidiazuron in the diet for at least 90 days.[a]

Observation	Dose (ppm)				
	0	200	600	1800	5400
Prostate Gland					
Diminished secretion	0	0	0	20	30
slight	0	0	0	10	20
mild	0	0	0	10	10
Adrenal Glands					
Hypertrophy, zona glomerulosa, diffuse	0	10	10	60	100
slight	0	10	10	60	80
mild	0	0	0	0	20
Kidney					
Glomerulopathy, vacuolation/mineralization	0	0	0	70	100
slight	0	0	0	70	40
mild	0	0	0	0	60
Hyperplasia, pelvic epithelium, bilateral	10	0	10	30	40
slight	10	0	10	20	20
mild	0	0	0	10	20
Mineralization, concretions, pelvis	0	0	0	20	30
slight	0	0	0	20	20
mild	0	0	0	0	10
Mineralization, inner medulla, focal, slight	0	0	10	10	30
Inflammation, mononuclear cell, focal, slight	0	10	0	30	70
Liver					
Hypertrophy, hepatocellular, centrilobular	0	0	0	30	90
slight	0	0	0	30	80
mild	0	0	0	0	10
Spleen					
Hyperplasia, germinal centers	0	0	0	50	100
slight	0	0	0	40	40
mild	0	0	0	10	60
Seminal Vesicle					
Diminished secretion	0	0	0	40	70
slight	0	0	0	0	60
mild	0	0	0	30	10
marked	0	0	0	10	0
Mammary Gland					
Atrophy, diffuse	0	0	0	20	90
slight	0	0	0	20	10

(table continues next page)

Observation	Dose (ppm)				
	0	200	600	1800	5400
mild	0	0	0	0	70
moderate	0	0	0	0	10
Germinal centers, inguinal lymph node	0	0	0	0	60
slight	0	0	0	0	50
mild	0	0	0	0	10
Thymus					
Germinal centers, medulla	0	0	0	20	50
slight	0	0	0	20	40
mild	0	0	0	0	10
Epididymis					
Inflammation, mononuclear cell, focal, slight	20	—	—	—	80
Bone and Marrow: Sternum					
Infiltration, adipose	10	0	0	10	90
slight	10	0	0	10	70
mild	0	0	0	0	20
Lung					
Alveolar macrophages, foamy, focal, slight	10	20	20	10	40
Hemorrhage, focal, slight	30	0	0	0	60
Lymph node, mesenteric					
Germinal centers	0	0	0	0	80
slight	0	0	0	0	70
mild	0	0	0	0	10
Testis					
Atrophy, seminiferous epithelium, unilateral	10	—	—	—	50
slight	0	—	—	—	50
marked	10	—	—	—	0

a Data were obtained from pages 133-142 in MRID 46121506.
— Not examined

(table continues next page)

Table 8b. Microscopic pathology findings (% incidence) in female rats treated with thidiazuron in the diet for at least 90 days.[a]

Observation	Dose (ppm)				
	0	200	600	1800	5400
Adrenal Gland					
Hypertrophy, zona glomerulosa, diffuse	0	10	40	60	100
slight	0	10	40	60	40
mild	0	0	0	0	60
Atrophy, zona reticularis	0	0	0	0	40
slight	0	0	0	0	10
mild	0	0	0	0	30
Pigment, brown, zona reticularis, slight	0	0	0	0	40
Kidney					
Glomerulopathy, vacuolation/mineralization	0	0	0	50	100
slight	0	0	0	50	80
mild	0	0	0	0	20
Basophilic tubules, focal, slight	0	0	0	20	30
Hyperplasia, pelvic epithelium, bilateral	10	10	10	10	60
slight	10	10	0	10	40
mild	0	0	10	0	20
Mineralization, concretions, pelvis, slight	20	0	10	0	40
Inflammation, mononuclear cell, focal, slight	0	0	0	0	30
Liver					
Hypertrophy, hepatocellular, centrilobular, slight	0	0	0	20	80
Bone and marrow: sternum					
Infiltration, adipose	60	60	50	90	100
slight	60	50	40	70	20
mild	0	10	10	20	50
moderate	0	0	0	0	30
Ovary					
Number of corpora lutea (recent)	70	100	90	100	20
1-5	30	70	80	90	20
6-10	40	30	10	10	0
Hypertrophy, interstitial gland	0	0	0	20	100
slight	0	0	0	20	30
mild	0	0	0	0	70
Spleen					
Hyperplasia, germinal centers	0	0	0	50	100
slight	0	0	0	50	10
mild	0	0	0	0	70
moderate	0	0	0	0	20
Uterus					

(table continues next page)

Observation	Dose (ppm)				
	0	200	600	1800	5400
No cyclical activity	0	0	0	0	60
Atrophy, diffuse	0	0	0	0	80
slight	0	0	0	0	10
mild	0	0	0	0	10
moderate	0	0	0	0	10
marked	0	0	0	0	50
Vagina					
Atrophy, diffuse	0	0	0	0	60
slight	0	0	0	0	10
mild	0	0	0	0	10
moderate	0	0	0	0	10
marked	0	0	0	0	30
Mammary Gland					
Atrophy, diffuse	0	0	0	0	90
slight	0	0	0	0	20
mild	0	0	0	0	70
Germinal centers, inguinal lymph node, slight	0	0	0	0	60
Lymph Node, mesenteric					
Germinal centers, slight	0	0	0	0	80
Femur: marrow and joint					
Atrophy, trabecular bone	0	0	0	0	50
slight	0	0	0	0	30
mild	0	0	0	0	20

a Data were obtained from pages 143-152 in MRID 46121506.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS: The NOAEL of thidiazuron is 200 ppm in males and females, based on hypertrophy of the zona glomerulosa in the adrenal of one male and four females and increased serum urea concentration in males. At 600 ppm, higher mean urea concentrations were noted for males only. Body weight gain from Day 1 to 90 was lower for males at 1800 ppm, and for both sexes at 5400 ppm. Food consumption at both of these levels was also significantly lower throughout the study. At 5400 ppm, piloerection, few feces, and thin appearance were noted for all animals for the first few weeks of treatment. At terminal sacrifice, increases in mean inorganic phosphorus (females only), cholesterol, urea, and potassium concentrations, alkaline phosphatase activity, and urinary volume (with a statistically significant trend towards a lower refractive index) were seen in both sexes at 5400 ppm. At 1800 and 5400 ppm, macroscopic and/or microscopic changes were observed in the adrenal gland, epididymis, heart, kidney, liver, mammary gland, ovary, pituitary gland, prostate, seminal vesicles, spleen, thymus, and uterus. In addition, microscopic changes were noted in bone marrow and vagina at 5400 ppm. At 16,200 ppm, all animals were found dead or moribund on Day 9 or 10. In all cases, it was after a marked body weight loss, a severe reduction of food consumption, and observation of various clinical signs of toxicity.

B. REVIEWER COMMENTS: No treatment-related effects were observed at 200 or 600 ppm, or on ophthalmology or hematology at any dose.

All ≥5400 ppm rats exhibited piloerection (first observed on Day 6), appeared thin (first observed on Day 4), and excreted few feces (first observed on Day 6). In addition, the 16,200 ppm males had a hunched posture (70% treated vs 0% controls; first observed on Day 8). No other clinical signs of toxicity were observed. Additionally at 16,200 ppm, all animals demonstrated deficits in all reflexes tested (righting, grasping, corneal, pupillary, head shaking, and auditory startle). All animals in this dose group were either found dead or were sacrificed moribund by Day 10.

Decreased ($p \leq 0.01$) body weights were observed throughout treatment in the ≥1800 ppm males (↓7-33%) and in the 5400 ppm females (↓15-26%). Overall (Days 1-90) body weight gains were also decreased ($p \leq 0.01$) in the ≥1800 ppm males (↓27-52%) and in the 5400 ppm females (↓43%). Decreased ($p \leq 0.05$) food consumption was generally observed throughout the study (↓11-66%) and overall (Days 1-90; ↓12-32%; $p \leq 0.01$) in the ≥1800 ppm males. Food consumption was also decreased ($p \leq 0.01$) in the 5400 ppm females (↓16-69%). No treatment-related effect was observed on body weights, body weight gains, or food consumption at 200 or 600 ppm.

Increased ($p \leq 0.05$) urea was observed in the 1800 ppm group (↑19-23%). Increased ($p \leq 0.01$) alkaline phosphatase was noted in the ≥1800 ppm males (↑31-39%) and 5400 ppm females (↑102%). In the 5400 ppm group, increased ($p \leq 0.01$) cholesterol (↑42-51%) and potassium (↑9-

16%) were observed. In the 5400 ppm females, increased ($p \leq 0.05$) phosphorus was noted (↑18%). Also, at 5400 ppm, urine volume was increased ($p \leq 0.01$) in both sexes (↑89-134%).

Decreased ($p \leq 0.01$) terminal body weights were observed in the ≥1800 ppm males (↓14-26%), and in the 5400 ppm females (↓15%). In the ≥1800 ppm males, decreases ($p \leq 0.05$) were noted in absolute epididymides weight (↓14-26%) and absolute and relative (to body) prostate gland weight (↓38-56%). Additionally in the 5400 ppm males, the following increases ($p \leq 0.01$) in relative organ weights were observed: (i) adrenal gland (↑25%); (ii) kidney (↑23%); (iii) liver (↑28%); and (iv) spleen (↑49%). Relative testes weight was decreased 33% ($p \leq 0.001$). In the 5400 ppm females, decreased ($p \leq 0.05$) were noted in absolute and relative adrenal gland (↓22-34%) and uterus (↓60-67%) weights. Increased ($p \leq 0.001$) relative kidney (↑22%) and liver (↑31%) weights were observed, and decreased ($p \leq 0.05$) absolute mesenteric lymph node weight (↓29%) was noted.

In the ≥1800 ppm males, small prostate was observed (30-80% treated vs 0% controls) and small seminal vesicles were noted (40-90% treated vs 0% controls). In the females, small uterus was observed at 5400 ppm (60% treated vs 0% controls). No other treatment-related findings were noted at necropsy.

In the ≥1800 ppm males, the following were observed: (i) slight to mild diminished secretion of the prostate gland (20-30% treated vs 0% controls); (ii) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland (60-100% treated vs 0% controls); (iii) slight to mild vacuolation/mineralization glomerulopathy of the kidney (70-100% treated vs 0% controls); (iv) slight to mild bilateral hyperplasia of the pelvic epithelium (30-40% treated vs 0% controls); (v) slight to mild mineralized concretions of the renal pelvis (20-30% treated vs 0% controls); (vi) slight focal mononuclear cell inflammation of the kidney (30-70% treated vs 0% controls); (vii) slight to mild centrilobular hepatocellular hypertrophy (30-90% treated vs 0% controls); (viii) slight to mild hyperplasia of germinal centers in the spleen (50-100% treated vs 0% controls); (ix) slight to marked diminished secretion of the seminal vesicle (40-70% treated vs 0% controls); (x) slight to moderate diffuse atrophy of the mammary gland (20-90% treated vs 0% controls); and (xi) slight to mild germinal centers in the medulla of the thymus (20-50% treated vs 0% controls).

In the ≥1800 ppm females, the following were noted: (i) slight to mild diffuse hypertrophy of the zona glomerulosa of the adrenal gland (60-100% treated vs 0% controls); (ii) slight to mild vacuolation/mineralization glomerulopathy of the kidney (50-100% treated vs 0% controls); (iii) slight centrilobular hepatocellular hypertrophy (20-80% treated vs 0% controls); (iv) slight to moderate adipose infiltration of the bone and marrow of the sternum (90-100% treated vs 60% controls); (v) slight to mild hypertrophy of the interstitial gland of the ovary (20-100% treated vs 0% controls); and (vi) slight to moderate hyperplasia of germinal centers in the spleen (50-100% treated vs 0% controls).

In the 5400 ppm males, the following were observed: (i) slight focal mononuclear cell inflammation of the epididymis (80% treated vs 20% controls); (ii) slight focal mineralization of the inner medulla of the kidney (30% treated vs 0% controls); (iii) slight to mild adipose infiltration of the bone and marrow of the sternum (90% treated vs 10% controls); (iv) slight focal foamy alveolar macrophages (40% treated vs 10% controls); (v) slight focal hemorrhage of the lung (60% treated vs 30% controls); (vi) slight to mild germinal centers of the mesenteric (80% treated vs 0% controls) and inguinal (60% treated vs 0% controls) lymph nodes; and (vii) slight unilateral atrophy of the seminiferous epithelium of the testes (50% treated vs 0% controls).

In the 5400 ppm females, the following were noted: (i) slight to mild atrophy of the zona reticularis of the adrenal gland (40% treated vs 0% controls); (ii) slight brown pigment in the zona reticularis of the adrenal gland (40% treated vs 0% controls); (iii) no cyclical activity of the uterus (60% treated vs 0% controls); (iv) slight to marked diffuse atrophy of the uterus (80% treated vs 0% controls); (v) slight to marked diffuse atrophy of the vagina (60% treated vs 0% controls); (vi) reduced numbers of recent corpora lutea in the ovary (20% treated vs 70% controls); (vii) slight to mild diffuse atrophy of the mammary gland (90% treated vs 0% controls); (viii) slight germinal centers of the mesenteric (80% treated vs 0% controls) and inguinal (60% treated vs 0% controls) lymph nodes; and (ix) slight to mild atrophy of the trabecular bone (50% treated vs 0% controls). All other microscopic findings were unaffected by treatment.

The LOAEL is 1800 ppm (equivalent to 102/123 mg/kg/day [M/F]), based on decreased body weights, overall (Days 1-90) body weight gains, and food consumption, increased serum alkaline phosphatase, decreased absolute epididymides and absolute and relative (to body) prostate gland weight, small prostate and small seminal vesicles, and microscopic findings in the prostate gland, seminal vesicle, mammary gland, and thymus of the males, microscopic findings in the bone and marrow of the sternum and ovary of the females, and increased urea and microscopic findings in the adrenal gland, kidney, liver, and spleen of both sexes. The NOAEL is 600 ppm (equivalent to 34.5/42.1 mg/kg/day [M/F]).

This study is classified as **acceptable/guideline** and satisfies the guideline requirements (OPPTS 870.3100a; OECD 408) for a subchronic oral toxicity study in the rat.

C. **STUDY DEFICIENCIES:** The following minor deficiencies were noted but do not change the conclusions of this review:

- A dose selection rationale was not provided.
- Sorbitol dehydrogenase was not measured.
- The nose and pharynx were not collected and examined.
- A summary table of ophthalmological findings was not included.

DATA FOR ENTRY INTO ISIS

Subchronic (90 day) Oral Study - rodents (870.3100)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
120301	46121506	subchronic	rat	90 days	oral	diet	11.2-325	0/0, 11.2/14.0, 34.5/42.1, 102/123, and 294/325 [M/F]	34.5	102	BW, BWG, FC, Clinical chemistry, Adrenal gland, Epididymides, Prostate gland, Seminal vesicle, Bone, Kidney, Liver, Mammary gland, Spleen, Thymus, Ovary	

8

DATA EVALUATION RECORD

THIDIAZURON

Study Type: §83-3b; Developmental Toxicity Study in Rabbits

Work Assignment No. 1-01-17 G (MRID 46121507)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature:** Paul Chi
Reregistration Branch 1, Health Effects Division (7509C) **Date** 11/16/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Why Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 11/18/04
Work Assignment Manager: P.V. Shah, Ph.D. **Signature:** Bhavi Dawman
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** Not provided

TEST MATERIAL (PURITY): Thidiazuron technical (99.5% a.i.)

SYNONYMS: 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea

CITATION: Wason, S. (2003) Thidiazuron: developmental toxicity study in the rabbit by gavage. Bayer CropScience, Sophia Antipolis Cedex, France. Laboratory Study No.: SA 02046, August 20, 2003. MRID 46121507. Unpublished.

Wason, S. (2004) Historical control data: Supplement to MRID 46121507: Thidiazuron–Developmental toxicity study in the rabbit by gavage. April 16, 2004. MRID 46252001. Unpublished.

Kennel, P. (2001). Thidiazuron: Range-finding study for developmental toxicity in rabbits. Aventis CropScience, Sophia Antipolis Cedex, France. Report of study SA 01070. July 19, 2001. MRID 46241001. Unpublished.

SPONSOR: Bayer AG, Bayer CropScience, Alfred Nobel Str. 50, Monheim, Germany

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 46121507), Thidiazuron (99.5% a.i.; Lot/Batch # 107623-03) in 0.5% (w/v) aqueous methylcellulose was administered daily by oral gavage at a dose volume of 4 mL/kg body weight to 25 female New Zealand White [KBL (NZW) lOPS/SPF] rabbits/group at dose levels of 0, 5, 25, or 125 mg/kg on gestation days (GD) 6 through 28. All does were sacrificed on GD 29; their fetuses were removed by cesarean and examined.

Maternal toxicity:

At 125 mg/kg/day, five pregnant females aborted between GD 27 and 29. These abortions were considered to be due to the test substance, either directly or indirectly (as a result of the marked decreases in food consumption and body weight). Red traces (8/25 treated vs 1/25 controls), placental tissue (4/25 treated vs 0/25 controls), and/or feto-placental tissue (5/25 treated vs 0/25 controls) found on the cage trays corresponded to the abortions. Other clinical signs of toxicity at this dose included increased incidence of soft/mucoid feces (1-3/25 treated vs 0/25 controls), few feces (14/25 treated vs 3/25 controls), and localized soiled fur (4/25 treated vs 0/25 controls).

Additionally at 125 mg/kg/day, cumulative body weight gains from GD 6 were decreased ($p<=0.05$) throughout treatment, resulting in decreased ($p<=0.01$) body weight gains for the overall (GD 6-29) treatment period, both uncorrected (decr. 70%) and corrected (decr. 171%) for gravid uterine weights. Gravid uterine weights of the treated groups were comparable to controls. Food consumption was decreased by 19-44% ($p<=0.01$) at this dose throughout treatment, except for during GD 26-29 which was decreased by 9% (not significant) compared to controls.

The only findings at 25 mg/kg/day were a single abortion thought to be due to maternal stress from gavage error (i.e., inflammation and red liquid in trachea) and clinical signs associated with abortion (i.e, red traces and feto-placental tissue on tray).

The maternal LOAEL is 125 mg/kg/day, based on abortions and decreased body weight gains and food consumption. The maternal NOAEL is 25 mg/kg/day.

Developmental toxicity

No effects of treatment were noted on numbers of litters, fetuses (live or dead), resorptions (early, late, or complete litter), sex ratio, or post-implantation loss.

At 125 mg/kg/day, fetal body weights were decreased ($p<=0.01$) by 12-13%, and the number of runts was increased compared to concurrent and historical controls. Fetal and/or litter incidences of the following variations, indicative of skeletal retardation, were increased at 125 mg/kg/day over concurrent and historical controls: (i) unilateral or bilateral holes in the frontal or parietal bones; (ii) unossification of the atlas centrum; (iii) unossification of the insertion point(s) on the pelvic girdle; and (iv) incomplete ossification or unossification of the pubis.

Aside from the above-mentioned skeletal retardations, there were no developmental variations that could definitely be attributed to treatment.

There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL is 125 mg/kg/day, based on decreased fetal body weights, increased number of runts, and delayed skeletal ossification. The developmental NOAEL is 25 mg/kg/day.

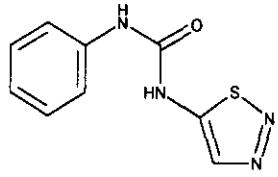
This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfies the requirements for a developmental study in the rabbit.

COMPLIANCE: Signed and dated Data Confidentiality, GLP, Flagging, and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Thidiazuron

Description:	Light yellow powder
Lot/Batch #:	107623-03
Purity:	99.5% a.i.
Compound Stability:	The test substance was stable in the vehicle for 28 days under conditions similar to the current study.
CAS #of TGAI:	51707-55-2
Structure:	

2. Vehicle and/or positive control: 0.5% (w/v) aqueous methylcellulose 400

3. Test animals:

Species:	Rabbit	
Strain:	New Zealand White [KBL (NZW) lOPS/SPF]	
Age/body weight range:	Approximately 18 weeks at receipt (GD 1 or 2)/3.02-3.89 kg on GD 3	
Source:	Elevage Scientifique des Dombes, Chatillon-sur-Chalaronne, France)	
Housing:	Individually in polycarbonate cages on a perforated cage floor	
Diet:	U.A.R #110C-10 laboratory animal pellets (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France), *ad libitum*	
Water:	Filtered, sterilized tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	17-21°C
	Humidity:	40-70%
	Air changes:	15/hour
	Photoperiod:	16 hrs light/8 hrs dark
Acclimation period:	4 or 5 days	

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: September 10, 2002 End: October 30, 2002

2. **Mating:** The females were naturally mated with breeder male rabbits of the same strain by the supplier prior to shipment. Rabbits were time-mated and arrived at the performing laboratory on gestation day (GD) 1 or 2. The day of insemination was designated as GD 0.

3. **Animal assignment:** The females were randomly assigned (blocked by day of mating) to the treatment groups indicated in Table 1. Body weight means were checked to ensure similar means among all groups. At study initiation, the body weight of each animal was within 20% of the mean body weight.

Table 1. Animal assignment [a]

Dose (mg/kg bw/day)	0	5	25	125
# Females	25	25	25	25

a Data obtained from page 15 of the study report.

4. **Dose selection rationale:** In a dose range-finding developmental toxicity study (MRID 46241001; SA 01070), Thidiazuron was administered daily by oral gavage to female rabbits at dose levels of 0, 10, 30, 100, or 300 mg/kg on GD 6 through 28. It was stated that the 300 mg/kg dose exceeded the maximum tolerated dose for pregnant rabbits. Body weights and food consumption were decreased at 100 mg/kg/day. No additional information was provided. For the current study, the dose level of 125 mg/kg/day was selected because it was expected to produce maternal toxicity similar to the 100 mg/kg/day group in the range-finding study. The dose level of 5 mg/kg/day was selected as the expected NOAEL.

5. **Dosage preparation and analysis:** The appropriate amount of test substance was periodically (six formulations) suspended in aqueous 0.5% (w/v) aqueous methylcellulose 400 and stored at approximately 5°C until use. Homogeneity of the suspensions was confirmed at the low and high concentration for the first formulation. Concentration analyses were performed on all formulations prepared for each dose group during the study. Stability of the test substance in the vehicle was confirmed previously in the range-finding study (SA 01070) for 28 days under conditions similar to those in the current study (i.e, dose formulations were stored at 5 ± 3°C and were removed from the refrigerator daily for four hours, during which time they were stirred at room temperature).

Results -

Homogeneity (range as % of nominal): 94-101%

Stability (range as % of nominal): 97-102% for up to 28 days under conditions similar to the current study.

Concentration (range as % nominal): 97-105%

The analytical data indicated that the mixing procedure was adequate and that the variation between nominal and actual dosage to the study animals was acceptable.

6. Dosage administration: Doses were administered once daily by oral gavage, on GDs 6-28, in a volume of 4 mL/kg of body weight. Dosing was adjusted based on each animal's most recently recorded body weight. Control animals received the vehicle alone. Dose suspensions were mixed continuously before and during treatment with an electromagnetic stirrer.

C. OBSERVATIONS

1. Maternal observations and evaluations: All does were checked for mortality, morbidity, and abortion twice daily during the week and once daily on weekends and holidays. Clinical signs of toxicity were recorded daily from GD 2 through 29. Body weights were measured on GD 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 29. Body weight gains were determined for the intervals between body weight measurements and for the overall (GD 6-29) treatment period, both corrected and uncorrected for gravid uterine weights. Food consumption (g/rabbit/day) was reported for GD 3-6, 6-8, 8-10, 10-14, 14-18, 18-22, 22-26, and 26-29. Rabbits sacrificed because of abortion, killed *in extremis*, or found dead were necropsied. The number and type of implantations and corpora lutea were noted when present. The number of ribs was also recorded. In cases in which corpora lutea were present but there were no visible implantations, the uterus was immersed in 20% ammonium sulfide to detect implantation scars. On GD 29, all surviving does were sacrificed and subjected to necropsy, the uteri excised, and gravid uterus weights determined. All fetuses were removed by cesarean section. The numbers of corpora lutea, implantations, resorptions (early and late), and fetuses (live and dead) were recorded.

2. Fetal evaluations: All live fetuses were weighed, examined externally, dissected for visceral examination, and sexed. The heads of approximately one-half of the fetuses in each litter were removed, fixed in Bouin's solution, and examined internally. All fetuses were then fixed in absolute ethanol, stained according to a modified Staples and Schnell technique, and examined for skeletal malformations, variations, and anomalies.

D. DATA ANALYSIS

1. Statistical analyses: Data were subjected to the following statistical procedures:

Parameter	Statistical test
Maternal body weight gains and corrected (for gravid uterine weight) body weight gain Numbers of corpora lutea, implantations, and resorptions (early and late).	Bartlett's test for homogeneity of variances. If variances were homogeneous (parametric analyses), analysis of variance (ANOVA) was performed, followed by pair-wise comparison of treated groups with controls using Dunnett's test (2-sided), if significant ANOVA. If variances were not homogeneous (non-parametric analyses), Kruskal-Wallis test was performed, followed by pair-wise comparison of treated groups with controls using Dunn's test (2-sided), if significant Kruskal-Wallis.
Maternal food consumption	The same procedures as above were used, except that a log transformation of the data was attempted to achieve homogeneity. If log transformed data continued to have variances that were not homogeneous, the non-parametric procedures listed above were followed.
Pre- and post-implantation losses	The same procedures as above were used, except that an arc-sine transformation of the data was attempted to achieve homogeneity. If the transformed data continued to have variances that were not homogeneous, the non-parametric procedures listed above were followed.
Fetal sex	Chi-square test
Live vs. dead fetuses (on both a fetal basis and a litter basis)	Fisher's exact test (2-sided)
Fetal body weight	Bartlett's test for homogeneity of variances. If variances were homogeneous, means were compared using a mixed linear model with the doe included as a random effect. If the F-test of fixed effect was significant, pair-wise comparison of treated groups with controls was conducted using Dunnett's test (2-sided). If variances were not homogeneous, Kruskal-Wallis test was performed, followed by pair-wise comparison of treated groups with controls using Dunn's test (2-sided), if significant Kruskal-Wallis

Significance was denoted at $p \leq 0.05$ or $p \leq 0.01$ for each comparison. The statistical procedures were considered appropriate.

2. <u>Indices</u>: The following indices were calculated from the cesarean section records of animals in the study:

Pre-implantation loss (%) = (# corpora lutea - # implantations)/# corpora lutea x 100

Post-implantation loss (%) = (# implantations - # viable fetuses)/# implantations x 100

Corrected body weight gain = body weight on GD 29 - body weight on GD 6 - gravid uterine weight

3. <u>Historical control data</u>: Historical control data were provided as a supplement to MRID 46121507 and included data from 3 recent developmental toxicity studies (dates not provided) conducted on rabbits by the same performing laboratory.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality: Five pregnant females in the 125 mg/kg/day group aborted between GD 27 and 29 and were subsequently sacrificed on the day they aborted. These abortions were considered due to the test substance, either directly or indirectly (as a result of the marked decreases in food consumption and body weight). There were no other abortions or mortalities that could be attributed to treatment. One doe at 25 mg/kg/day aborted on GD 21. Macroscopic evaluation of this animal at necropsy revealed inflammation and red liquid in the trachea, likely due to gavage error. One control doe was found dead on GD 14, after having clonic convulsions and soiling around the mouth following dosing. Gross necropsy of this animal revealed foam in the lungs and trachea, indicating gavage error. There were no mortalities or abortions at 5 mg/kg/day.

2. Clinical observations: At 125 mg/kg/day, there was an increased incidence of does with soft/mucoid feces (1-3/25 does vs 0/25 controls), few feces (14/25 does vs 3/25 controls), and localized soiled fur (4/25 treated vs 0/25 controls; Table 2). At ≥25 mg/kg/day, red traces were found on the tray (4-8/25 treated vs 1/25 controls). Placental and/or feto-placental tissue was found on the cage trays of the 25 mg/kg does (1/25 treated vs 0/25 controls) and the 125 mg/kg does (4-5/25 treated) and corresponded to the abortions at 25 mg/kg (1) and 125 mg/kg (5).

Table 2. Clinical signs of toxicity [# of does affected][a]

Clinical sign		Dose in mg/kg bw/day (# of Dams)			
		0 (25)	5 (25)	25 (25)	125 (25)
Abortion		0	0	1	5
Feces -	soft	0	0	1	3
	mucoid	0	0	0	1
	few	3	1	1	14
Localized soiled fur		0	0	0	4
Found on tray -	red traces	1	0	4	8
	placental tissue	0	0	0	4
	feto-placental tissue	0	0	1	5

a Data obtained from Tables 1 and 2 on pages 40-43 of the study report.

3. Body weight:

Absolute mean body weights of the low- and mid-dose groups were comparable to those of the controls at various measuring intervals. The body weights of high dose group showed a slight decrease with no statistical significance. At 125 mg/kg/day, cumulative body weight gains from GD 6 were decreased ($p \leq 0.05$) throughout treatment, resulting in decreased ($p \leq 0.01$) body weight gains for the overall (GD 6-29) treatment period, both uncorrected (↓70%) and corrected

(↓171%) for gravid uterine weights (Table 3). Gravid uterine weights of the treated groups were comparable to controls.

Table 3. Mean (±SD) maternal body weight gain (kg)[a]

Interval		Dose in mg/kg bw/day (# of Does)			
		0 (22-23)	5 (24)	25 (24-25)	125 (20-25)
Pretreatment:	GD 3-6	0.03 ± 0.093	0.06 ± 0.079	0.02 ± 0.084	0.01 ± 0.054
Treatment	GD 6-29	0.33 ± 0.131	0.34 ± 0.113	0.33 ± 0.113	0.10 ± 0.283** (↓70)
	Corrected	-0.14 ± 0.115	-0.14 ± 0.143	-0.16 ± 0.106	-0.38 ± 0.234** (↓171)
Gravid uterus weight [b]		0.47	0.48	0.49	0.48

a Data obtained from Table 3 on pages 48-49 of the study report. Percent difference from controls (calculated by reviewers) is included in parentheses.

b Gravid uterine weights were calculated by the reviewers from data presented in this table.

** Significantly different from controls, p≤0.01

4. **Food consumption:** At 125 mg/kg/day, food consumption was decreased (↓19-44%; p≤0.01) throughout treatment, except for during GD 26-29 which was decreased by 9% (not significant) compared to controls (Table 4). There were no other effects of treatment on food consumption.

Table 4. Mean (±SD) food consumption[a]

Interval		Dose in mg/kg bw/day (# of Does)			
		0 (21-23)	5 (24)	25 (24-25)	125 (18-25)
Pretreatment:	GD 3-6	175.9 ± 25.20	180.0 ± 17.14	172.6 ± 25.95	170.3 ± 19.62
Treatment:	GD 6-8	170.2 ± 34.86	166.7 ± 29.48	162.3 ± 25.36	137.3 ± 24.75** (↓19)
	GD 14-18	165.3 ± 30.84	156.0 ± 29.71	153.0 ± 30.14	92.7 ± 44.03** (↓44)

a Data obtained from Table 4 on pages 51-52 of the study report. Percent difference from controls (calculated by reviewers) is included in parentheses.

** Significantly different from controls, p≤0.01

5. **Gross pathology:** There were no treatment-related macroscopic findings in any group.

6. **Cesarean section data:** Cesarean section data are presented in Table 5. Fetal body weights were decreased by 12-13% (p≤0.01) at 125 mg/kg/day. No effects of treatment were noted on numbers of litters, fetuses (live or dead), resorptions (early, late, or complete litter), sex ratio, or postimplantation loss.

Table 5. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	5	25	125
# Animals Assigned (Mated)	25	25	25	25
# Animals Pregnant	23	24	25	25
Pregnancy Rate (%)	92	96	100	100
# Nonpregnant	2	1	0	0
Maternal Wastage				
# Died	1	0	0	0
# Died Pregnant	1	0	0	0
# Died Nonpregnant	0	0	0	0
# Aborted	0	0	1	5
# Premature Delivery	0	0	0	0
Total # Corpora Lutea [b]	285	294	300	251
Corpora Lutea/Doe	13.0 ± 1.8	12.3 ± 1.6	12.5 ± 1.8	12.6 ± 2.5
Total # Implantations [b]	207	249	247	217
(Implantations/Doe)	9.4 ± 2.2	10.4 ± 2.6	10.3 ± 2.4	10.9 ± 2.6
Total # Litters	22	24	24	20
Total # Live Fetuses	200	220	230	196
(Live Fetuses/Doe)	9.1 ± 2.4	9.2 ± 2.7	9.6 ± 2.7	9.8 ± 2.9
Total # Dead Fetuses	2	10*	3	7
(Dead Fetuses/Doe) [c]	0.09	0.42	0.13	0.35
Total # Resorptions [c]	5	19	14	14
Early [b]	4	19	14	10
Late [b]	1	0	0	4
Total Resorptions/Doe [c]	0.23	0.79	0.58	0.70
Early	0.2 ± 0.7	0.8 ± 1.3*	0.6 ± 0.8	0.5 ± 0.8
Late	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.5
Complete Litter Resorption	0	0	0	0
Mean Fetal Weight (g)	36.2 ± 6.3	35.3 ± 6.3	34.9 ± 6.6	31.7 ± 6.3** (↓12)
Males	36.7 ± 6.0	34.7 ± 6.0	36.2 ± 6.1	32.2 ± 6.2** (↓12)
Females	35.6 ± 6.5	35.8 ± 6.6	34.0 ± 6.8	31.1 ± 6.4** (↓13)
Sex Ratio (% Male)	51.5 ± 14.7	44.6 ± 16.1	41.5 ± 17.1*	49.5 ± 17.8
Preimplantation Loss (%)	26.7 ± 16.2	15.4 ± 17.4*	18.2 ± 12.4	12.8 ± 15.7*
Postimplantation Loss (%)	4.1 ± 11.0	12.4 ± 12.3	7.4 ± 9.9	10.4 ± 14.0

a Data obtained from Table 1 on page 40 and Table 6 on pages 56-58 of the study report.
b Tabulated by the reviewers from individual data presented in Appendix E on pages 116-123 of the study report.
c Calculated by the reviewers from data presented in this table.
* Significantly different from controls at $p \leq 0.05$
** Significantly different from controls at $p \leq 0.01$

B. DEVELOPMENTAL TOXICITY

1. External examination: External findings are presented in Table 6a. The number of runts was increased at 125 mg/kg/day (24.4% fetuses; 70.0% litters) compared to concurrent (9.2% fetuses; 36.4% litters) and historical (5.5-16.3% fetuses; 36.4-54.2% litters) controls.

Fetal and litter incidences of malrotated forepaw(s) were increased at 125 mg/kg/day (2.1% fetuses; 10.0% litters) compared to concurrent (0.3% fetuses; 4.5% litters) and historical (0.0-1.3% fetuses; 0.0-8.3% litters) controls. However, it is unlikely that this anomaly is due to the test substance because the dose-relationship is tenuous at best. Furthermore, although the incidence at the high dose in the current study exceeded the range of historical controls, this anomaly is fairly common in control animals; and it is important to note that the historical control data was based on a small number of studies (i.e., 3). All other external findings in the fetuses were considered unrelated to treatment because they were not dose-related and/or because fetal and/or litter incidences fell within the range of historical controls.

Table 6a. External findings [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)				Historical Controls [b]
	0	5	25	125	
# Fetuses (# litters) examined	200 (22)	220 (24)	230 (24)	196 (20)	
Variations					
Runt (body weight <28 g)	9.2 (36.4)	12.4 (62.5)	11.6 (45.8)	24.4 (70.0)	5.5-16.3 (36.4-54.2)
Anomalies					
Forepaw(s) - malrotated outward/inward, unilateral/bilateral	0.3 (4.5)	0.5 (4.2)	0.0 (0.0)	2.1 (10.0)	0.0-1.3 (0.0-8.3)
Hindlimbs - hyperflexion, bilateral	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.5 (5.0)	0.0-1.5 (0.0-8.3)
Malformations					
Spina bifida	0.0 (0.0)	0.4 (4.2)	0.0 (0.0)	0.0 (0.0)	0.0-1.0 (0.0-4.2)

a Data were obtained from Table 7 on page 61 of the study report.
b Historical control data were obtained from pages 5-25 of a supplement to MRID 46121507.

2. Visceral examination: Selected visceral abnormalities are presented in Table 6b. Fetal and litter incidences of small gall bladder were observed at 125 mg/kg/day (2.8% fetuses; 20.0% litters) compared to concurrent (0.7% fetuses; 9.1% litters) and historical (0.0-1.3% fetuses; 0.0-12.5% litters) controls. Partial membranous ventricular wall was noted at 125 mg/kg/day (1.6% fetuses; 10.0% litters) and was not observed in the concurrent or historical controls. All other visceral variations, anomalies, and malformations were considered unrelated to treatment because they were incidental, were within the range of historical controls, and/or were not dose-related.

Table 6b. Selected visceral findings [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)				Historical Controls [b]
	0	5	25	125	
# Fetuses (# litters) examined	200 (22)	220 (24)	230 (24)	196 (20)	
Variations					
Innominate artery - short	1.0 (9.1)	0.4 (4.2)	2.6 (8.3)	2.8 (15.0)	1.0-3.9 (9.1-12.5)
Gall bladder - small	0.7 (9.1)	1.7 (16.7)	0.6 (4.2)	2.8 (20.0)	0.0-1.3 (0.0-12.5)
Anomalies					
Carotid - narrowed, left	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.3 (5.0)	Not observed
Ventricle - membranous wall, partial	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.6 (10.0)	Not observed
Kidney - malpositioned and/or short ureter, unilateral	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	1.3 (5.0)	0.0-1.4 (0.0-8.3)
renal pelvis dilated, unilateral	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.4 (5.0)	Not observed
Testes - malpositioned and/or misshapen, unilateral/bilateral	0.0 (0.0)	0.0 (0.0)	0.3 (4.2)	0.3 (5.0)	Not observed
Malformations					
Aorta - ascending, descending, and/or aortic arch - dilated and/or narrowed pulmonary trunk and/or ventricular septum defect in cranial region and/or enlarged left ventricle and/or small right ventricle	1.8 (13.6)	0.4 (4.2)	0.0 (0.0)	2.1 (15.0)	0.0-1.4 (0.0-12.5)
Diaphragmatic hernia and all lung lobes small	0.0 (0.0)	0.0 (0.0)	0.4 (4.2)	0.0 (0.0)	Not observed

a Data were obtained from Table 8 on pages 63-64 of the study report..
b Historical control data were obtained from pages 18-23 of a supplement to MRID 46121507.

3. <u>Skeletal examination</u>: Selected skeletal findings are presented in Table 6c. Fetal and/or litter incidences of the following variations, indicative of skeletal retardation, were increased over concurrent and/or historical controls: (i) unilateral or bilateral holes in the frontal or parietal bones at 125 mg/kg/day (3.3% fetuses, 10.0% litters) compared to concurrent (0.6% fetuses, 4.5% litters) and historical (0.6-1.5% fetuses, 4.2-8.3% litters) controls; (ii) unossification of the atlas centrum at 125 mg/kg/day (1.5% fetuses, 15.0% litters) compared to concurrent (0.5% fetuses, 4.5% litters) and historical (0.0-4.3% fetuses, 0.0-12.5% litters); (iii) unossification of the insertion point(s) on the pelvic girdle at 125 mg/kg/day (3.9% fetuses, 10.0% litters) compared to concurrent (0% fetuses, 0% litters) and historical (0.0-2.1% fetuses, 0.0-12.5% litters); (iv) incomplete ossification of the pubis at ≥25 mg/kg/day (5.4-16.1% fetuses, 37.5-65.0% litters) compared to concurrent (3.1% fetuses, 27.3% litters) and historical (0.8-3.1% fetuses, 8.3-27.3% litters); and (v) unossification of the pubis at ≥25 mg/kg/day (1.0-1.3% fetuses, 10.0-12.5% litters) compared to concurrent (0.5% fetuses, 4.5% litters) and historical (0.3-1.2% fetuses, 4.2-8.3% litters). There were no treatment-related skeletal anomalies or malformations.

Table 6c. Selected skeletal findings [% fetuses affected (% litters affected)][a]

Observations	Dose (mg/kg bw/day)				Historical Controls [b]
	0	5	25	125	
# Fetuses (# litters) examined	200 (22)	220 (24)	230 (24)	196 (20)	
Variations					
Frontal or parietal - holes, unilateral/bilateral	0.6 (4.5)	0.0 (0.0)	1.8 (8.3)	3.3 (10.0)	0.6-1.5 (4.2-8.3)
Atlas centrum - unossified	0.5 (4.5)	0.0 (0.0)	1.0 (8.3)	1.5 (15.0)	0.0-4.3 (0.0-12.5)
Pelvic girdle - insertion point(s) unossified	0.0 (0.0)	0.9 (8.3)	0.9 (8.3)	3.9 (10.0)	0.0-2.1 (0.0-12.5)
insertion point(s) on 2nd sacral vertebra	31.0 (81.8)	30.0 (87.5)	37.3 (83.3)	45.7 (90.0)	31.0-56.2 (81.8-95.8)
Pubis - incomplete ossification	3.1 (27.3)	4.7 (20.8)	5.4 (37.5)	16.1 (65.0)	0.8-3.1 (8.3-27.3)
unossified	0.5 (4.5)	0.0 (0.0)	1.3 (12.5)	1.0 (10.0)	0.3-1.2 (4.2-8.3)
Anomalies					
Caudal vertebrae - one or two malpositioned	1.0 (9.1)	0.0 (0.0)	0.7 (8.3)	1.4 (10.0)	0.0-1.8 (0.0-12.5)
Malformations					
Thoracic region - one vertebra unossified; one or two ribs unossified or fused; extra rib or one rib detached or short; one or two arches unossified, incompletely ossified, or fused; one or two centrum unossified, hemicentric, misshapen, or incomplete	0.9 (9.1)	0.0 (0.0)	1.1 (8.3)	0.7 (5.0)	---
Lumbar arches - two fused, unilateral	0.0 (0.0)	0.4 (4.2)	0.0 (0.0)	0.0 (0.0)	Not observed

a Data obtained from Table 9 on pages 66-68 of the study report.
b Historical control data were obtained from pages 23-25 of a supplement to MRID 46121507.
--- Not observed in historical controls as this multiple malformation, although separate malformations were noted.

III. DISCUSSION and CONCLUSIONS

A. <u>INVESTIGATORS' CONCLUSIONS</u>: It was concluded that the maternal LOAEL was 25 mg/kg/day based on an isolated incidence of soft feces and three dams which showed red traces on the cage tray between GD 24 and 29. The investigators determined the developmental LOAEL to be 125 mg/kg/day based on a slight retardation in fetal maturation.

B. REVIEWER COMMENTS

1. Maternal toxicity: At 125 mg/kg/day, five pregnant females aborted between GD 27 and 29. These abortions were considered due to the test substance, either directly or indirectly (as a result of the marked decreases in food consumption and body weight). There were no other abortions or mortalities that could be attributed to treatment. Clinical signs of toxicity at this dose included increased incidence of soft/mucoid feces (1-3/25 treated vs 0/25 controls), few feces (14/25 treated vs 3/25 controls), and localized soiled fur (4/25 treated vs 0/25 controls).

Additionally at 125 mg/kg/day, cumulative body weight gains from GD 6 were decreased ($p \leq 0.05$) throughout treatment, resulting in decreased ($p \leq 0.01$) body weight gains for the overall (GD 6-29) treatment period, both uncorrected (↓70%) and corrected (↓171%) for gravid uterine weights. Gravid uterine weights of the treated groups were comparable to controls. Food consumption was decreased (↓19-44%; $p \leq 0.01$) at this dose throughout treatment, except for during GD 26-29 which was decreased by 9% (not significant) compared to controls.

The only findings at 25 mg/kg/day were a single abortion thought to be due to maternal stress from gavage error (i.e., inflammation and red liquid in trachea) and clinical signs associated with abortion (i.e, red traces and feto-placental tissue on tray).

The maternal LOAEL is 125 mg/kg/day, based on abortions and decreased body weight gains and food consumption. The maternal NOAEL is 25 mg/kg/day.

2. Developmental toxicity

a. Deaths/Resorptions: No effects of treatment were noted on numbers of litters, fetuses (live or dead), resorptions (early, late, or complete litter), sex ratio, or post-implantation loss.

b. Altered Growth: At 125 mg/kg/day, fetal body weights were decreased ($p<=0.01$) by 12-13%, and the number of runts was increased compared to concurrent and historical controls. Fetal and/or litter incidences of the following variations, indicative of skeletal retardation, were increased at 125 mg/kg/day over concurrent and historical controls: (i) unilateral or bilateral holes in the frontal or parietal bones; (ii) unossification of the atlas centrum; (iii) unossification of the insertion point(s) on the pelvic girdle; and (iv) incomplete ossification or unossification of the pubis.

The incidences of incomplete or unossification of the pubis at 25 mg/kg/day were not considered to be toxicologically important, because they were not corroborated by decreases in fetal body weights at this dose. No treatment related effects were reported at 25 mg/kg.

c. Developmental Variations: Aside from the above-mentioned skeletal retardations, there were no developmental variations that could definitely be attributed to treatment.

d. Malformations: There were no treatment-related external, visceral, or skeletal malformations.

The developmental LOAEL is 125 mg/kg/day, based on decreased fetal body weights, increased number of runts, and delayed skeletal ossification. The developmental NOAEL is 25 mg/kg/day.

This study is classified **acceptable/guideline (OPPTS 870.3700b)** and satisfies the requirements for a developmental study in the rabbit.

C. STUDY DEFICIENCIES: The following deficiency was noted but does not alter the conclusions of this DER:

- Body weights were not reported prior to GD 3.

DATA FOR ENTRY INTO ISIS

Developmental Study - rats (870.3700a)

PC code	MRID #	Study type	Species	Duration	Route	Dosing method	Dose range mg/kg/day	Doses tested mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ(s)	Comments
120301	46121507	developmental	rabbit	GD 6-28	oral	gavage	5-125	0, 5, 25, 125	25	125	Abortion Decr. BW, FC	Maternal
120301	46121507	developmental	rabbit	GD 6-28	oral	gavage	5-125	0, 5, 25, 125	25	125	Decr. fetal BW Incr. # of runts Delayed ossification	Developmental

9

DATA EVALUATION RECORD

THIDIAZURON

Study Type: §84-2; Bacterial Reverse Gene Mutation Assay

Work Assignment No. 1-01-17 H (MRID 46121508)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Date: 03/18/04

Quality Assurance:
Steven Brecher, Ph. D

Signature: Steven Brecher
Date: 3/18/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature**: Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 6/11/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/23/04
Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** Shazi Dawman
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Bacterial Gene Mutation (*Salmonella typhimurium* and *Escherichia coli*)/ Mammalian Activation Gene Mutation Assay; OPPTS 870.5100 [§84-2]; OECD 471 (formerly OECD 471 & 472).

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Kitching, J. (2001) Thidiazuron (code: AE B049537 00 1D990002): Bacterial mutation assay. Huntingdon Life Sciences, Ltd, Alconbury, Huntingdon, UK. Laboratory Project ID: AES 036, January 29, 2001. MRID 46121508. Unpublished.

SPONSOR: Aventis CropScience UK, Ltd., Toxicology, Chesterford Park, Saffron Walden, Essex, UK

EXECUTIVE SUMMARY - In two independent reverse gene mutation assays in bacteria (MRID 46121508), *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 *uvr*A/pKM101 (CM891) were exposed to Thidiazuron (98.7% purity, Batch/Lot #: CH 107623-02) in dimethylsulfoxide (DMSO) at concentrations of 0, 5, 15, 50, 150, 500, 1500, or 5000 µg/plate (±S9, Trial 1), and 0, 1.5, 5, 15, 50, 150, 500, or 1500 µg/plate (±S9, Trial 2). The standard plate incorporation method was performed in Trial 1 and a pre-incubation step was added in Trial 2. Standard strain-specific mutagens served as positive controls.

Thidiazuron was tested at up to the limit dose (5000 µg/plate) in Trial 1, and up to cytotoxic concentrations (1500 µg/plate) in Trial 2. Cytotoxicity (indicated by an incomplete background lawn) was observed in all strains at >=1500 µg/plate in Trial 1 (plate incorporation test), and in all strains at >=500 µg/plate in Trial 2 (pre-incubation test). No treatment-related increases in the number of revertants/plate were observed in any bacterial strain at any dose level of Thidiazuron in the presence or absence of S9-activation, compared to solvent controls. The

positive controls induced the appropriate responses. **There was no evidence of induced mutant colonies over background under the conditions of these tests.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

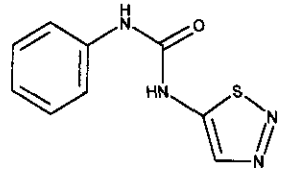
COMPLIANCE - Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description: Yellowish powder
Batch/Lot#: CH107623-02
Purity (w/w): 98.7% a.i.
Stability of compound: Not reported
CAS # of TGAI: 51707-55-2
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)

2. Control materials

Negative - The solvent alone served as the negative control.
Solvent - DMSO (0.1 mL/plate, plate incorporation and pre-incubation)
Positive

Non-activation

Sodium azide	0.5 µg/plate TA100, TA1535
2-Nitrofluorene	1.0 µg/plate TA98
9-Aminoacridine	30.0 µg/plate TA1537
2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide	0.05 µg/plate WP2 *uvr*A/pKM101 (CM891)

Activation

2-Aminoanthracene	2.0 µg/plate TA1535
	10.0 µg/plate WP2 *uvr*A/pKM101 (CM891)
Benzo[a]pyrene	5.0 µg/plate TA98, TA100, TA1537

Note: All positive controls were prepared in DMSO.

3. **Activation** - The S9 fraction was prepared by the performing laboratory and was derived from male Sprague-Dawley rats weighing <300 g:

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			None		Hamster		Other (name)
			Other (name)		Other (name)		

The S9 fraction was stored at -80°C prior to use. The S9 fraction was mixed (1:9, v/v) with the following cofactors to make the S9 mix: glucose-6-phosphate (5 mM), NADPH (4 mM), NADH (4 mM), $MgCl_2$ (8 mM), KCl (33 mM), and sodium phosphate buffer (100 mM at pH 7.4). The final S9 culture concentration was approximately 2%. The S9-mix was checked for sterility and efficacy.

4. **Test organisms**

S. typhimurium strains

	TA97	X	TA98	X	TA100		TA102		TA104
X	TA1535	X	TA1537		TA1538		Other		

E. coli strains

X	WP2*uvr*A/pKM101		WP2*uvr*A

Properly maintained? **Yes**
Checked for appropriate genetic markers (*rfa* mutation, R factor)? **Yes**

5. **Test compound concentrations used**

Preliminary cytotoxicity assay - Not performed

Mutagenicity assay - Tester strains TA98, TA100, TA1535, TA1537, and WP2*uvr*A/pKM101.
Non-activated conditions: 0, 5, 15, 50, 150, 500, 1500, or 5000 µg/plate (Trial 1, plate test)
0, 1.5, 5, 15, 50, 150, 500, or 1500 µg/plate (Trial 2, pre-incubation)
Activated conditions: 0, 5, 15, 50, 150, 500, 1500, or 5000 µg/plate (Trial 1, plate test)
0, 1.5, 5, 15, 50, 150, 500, or 1500 µg/plate (Trial 2, pre-incubation)

All concentrations of the test article, solvent, and positive controls were plated in triplicate, both in the presence and absence of S9-activation, for each tester strain (*S. typhimurium* TA98, TA100, TA1535, and TA1537 and *E. coli* WP2*uvr*A/pKM101).

B. TEST PERFORMANCE

1. **Type of assay**

x standard plate test (Trial 1)
x pre-incubation (30 minutes, Trial 2)
_ "Prival" modification *(i.e. azo-reduction method)*

_ spot test
_ other

2. Protocol - Two independent mutagenicity trials were conducted both in the presence and absence of S9. The standard plate incorporation method was used for Trial 1 and a pre-incubation step was performed in Trial 2. Prior to plating, inocula of the tester strains were cultured in nutrient broth (Merck No. 2) in a shaker/incubator for 10 hours at 37°C. In Trial 1, the test compound, solvent, or positive control (0.1 mL), bacteria (0.1 mL), and 0.5 mL of S9 mix (for tests requiring metabolic activation) or sodium phosphate buffer were placed in glass bottles, and 2.0 mL of melted top agar supplemented with 0.5 mM histidine/biotin/tryptophan were added. The top agar components were mixed and poured into triplicate plates containing 25 mL of solidified minimal agar. After solidification, the plates were incubated in the dark for approximately 72 hours at 37°C. After incubation, the plates were scored for number of revertant colonies using a Seescan automated colony counter. The plates were also checked for signs of cytotoxicity (thinning of the background lawn).

In the pre-incubation assay (Trial 2), the bacteria; test compound, solvent, or positive control; and S9 mix or buffer were mixed together and incubated in a shaker/incubator for 30 minutes at 37°C prior to addition of the 2.0 mL melted top agar. The plating and scoring proceeded as for the plate incorporation test described above.

3. Evaluation criteria

Assay validity - The assay was considered valid if the following criteria were met:

- The mean solvent control values were within the 99% confidence limits of the historical control range for each strain.
- The positive controls induced at least a doubling in revertant colonies compared to the concurrent solvent controls.

Positive result - The test article was considered to be mutagenic if the following criteria were met:

- A dose-related and reproducible increase in the number of revertant colonies (at least 2x the solvent controls) in at least one tester strain (±S9) was observed.

4. Statistical analysis - The means and standard deviations were calculated for each set of data. No other statistical evaluations were performed.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY - A preliminary cytotoxicity assay was not performed.

B. MUTAGENICITY ASSAY - The results of the mutagenicity trials were summarized in the study report Tables 1-4 on pages 20-25. As there were no positive results in either trial, only a representative table is included as an Attachment to this DER. In Trial 1 (plate incorporation test), cytotoxicity (indicated by an incomplete background lawn) was observed in all strains at ≥1500 µg/plate. No marked increases in revertant colonies were observed at any dose level in any strain (±S9). In Trial 2 (pre-incubation test), cytotoxicity (indicated by an incomplete background lawn) was observed in all strains at ≥500 µg/plate. No marked increases in revertant colonies were observed at any dose level in any strain (±S9). The positive controls induced marked increases in revertant colonies compared to controls in both trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that under the conditions of this study, Thidiazuron did not induce mutations in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 or *E. coli* WP2*uvr*A/pKM101 when tested up to 5000 µg/plate in the presence or absence of S9-activation.

B. REVIEWER COMMENTS - No treatment-related increases in revertant colonies were observed at any dose in any strain in either trial, in the presence or absence of S9-activation. The positive controls induced the appropriate responses. **There was no evidence of induced mutant colonies over background.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5100; OECD 471) for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

C. STUDY DEFICIENCIES - The following minor deficiency was noted, but does not change the conclusions of this DER:

- Dose formulations were not analyzed for actual concentrations.

ATTACHMENT

THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
SEE THE FILE COPY

TABLE 1

Mutation Test 1

Thidiazuron - revertant colony counts obtained per plate using bacterial strains TA1535, TA1537 and TA98

Strain	Dose level (µg/plate)	Liver S9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 1535	5000	-	-	-	IL, IL, IL
	1500	-	-	-	IL, IL, IL
	500	-	16	2	18, 15, 14
	150	-	21	4	17, 25, 20
	50	-	13	2	14, 11, 15
	15	-	15	5	21, 13, 11
	5	-	13	3	16, 10, 13
	Solvent	-	14	4	14, 10, 17
	5000	+	-	-	IL, IL, IL
	1500	+	-	-	IL, IL, IL
	500	+	14	2	14, 13, 16
	150	+	18	5	21, 20, 12
	50	+	11	1	12, 11, 11
	15	+	12	1	11, 13, 13
	5	+	13	2	14, 14, 11
	Solvent	+	19	3	16, 21, 19
TA 1537	5000	-	-	-	IL, IL, IL
	1500	-	-	-	IL, IL, IL
	500	-	8	3	5, 11, 7
	150	-	12	5	7, 17, 12
	50	-	10	2	11, 8, 11
	15	-	12	2	13, 10, 12
	5	-	10	4	8, 14, 8
	Solvent	-	12	4	7, 15, 13
	5000	+	-	-	IL, IL, IL
	1500	+	-	-	IL, IL, IL
	500	+	8	3	9, 4, 10
	150	+	16	4	19, 12, 17
	50	+	11	5	6, 10, 16
	15	+	13	3	16, 10, 14
	5	+	8	1	9, 8, 8
	Solvent	+	15	3	12, 17, 15
TA 98	5000	-	-	-	IL, IL, IL
	1500	-	-	-	IL, IL, IL
	500	-	23	3	20, 22, 26
	150	-	28	6	25, 25, 35
	50	-	25	2	23, 25, 27
	15	-	26	2	27, 27, 24
	5	-	27	5	25, 33, 23
	Solvent	-	26	4	29, 21, 28
	5000	+	-	-	IL, IL, IL
	1500	+	-	-	IL, IL, IL
	500	+	28	3	31, 26, 27
	150	+	24	1	24, 25, 24
	50	+	30	3	27, 29, 33
	15	+	23	2	22, 22, 26
	5	+	23	5	19, 21, 29
	Solvent	+	28	6	33, 29, 22

– Absence + Presence SD Standard deviation IL Incomplete bacterial lawn

TABLE 1

(continued)

Mutation Test 1

Thidiazuron - revertant colony counts obtained per plate using bacterial strains TA100 and WP2*uvr*A/pKM101 (CM891)

Strain	Dose level (µg/plate)	Liver S9	Mean revertant colony counts	SD	Individual revertant colony counts
TA 100	5000	-	-	-	IL, IL, IL
	1500	-	-	-	IL, IL, IL
	500	-	110	18	92, 127, 112
	150	-	119	8	117, 112, 128
	50	-	118	3	115, 119, 120
	15	-	105	7	112, 99, 105
	5	-	111	2	112, 111, 109
	Solvent	-	121	5	116, 121, 126
	5000	+	-	-	IL, IL, IL
	1500	+	-	-	IL, IL, IL
	500	+	93	4	96, 89, 95
	150	+	124	4	127, 125, 119
	50	+	122	4	127, 119, 120
	15	+	119	11	116, 110, 132
	5	+	105	11	93C, 115, 108
	Solvent	+	127	4	122, 129, 130
CM 891	5000	-	-	-	IL, IL, IL
	1500	-	-	-	IL, IL, IL
	500	-	99	18	94, 119, 84
	150	-	176	5	170, 178, 180
	50	-	187	17	207, 174, 181
	15	-	201	10	208, 205, 189
	5	-	201	20	216, 179, 209
	Solvent	-	215	9	209, 226, 211
	5000	+	-	-	IL, IL, IL
	1500	+	-	-	IL, IL, IL
	500	+	127	10	136, 117, 129
	150	+	204	17	187, 220, 205
	50	+	203	5	198, 208, 202
	15	+	223	4	222, 219, 227
	5	+	218	9	211, C, 224
	Solvent	+	194	12	208, 187, 186

\- Absence + Presence SD Standard deviation
C Contaminated IL Incomplete bacterial lawn

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DATA EVALUATION RECORD

THIDIAZURON

Study Type: §84-2; *In Vitro* Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes

Work Assignment No. 1-01-17 I (MRID 46121510)

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Date: 3/18/04

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

EPA Reviewer: Paul Chin **Signature**: Paul Chin
Reregistration Branch 1, Health Effects Division (7509C) **Date** 6/1/04
EPA Secondary Reviewer: Whang Phang, Ph.D. **Signature:** Whang Phang
Reregistration Action Branch 1, Health Effects Division (7509C) **Date** 9/27/04
Work Assignment Manager: P.V. Shah, Ph. D. **Signature**: Shari Dawson
Registration Action Branch 1, Health Effects Division (7509C) **Date** 12/10/04
Template version 11/01

DATA EVALUATION RECORD

STUDY TYPE: *In vitro* Mammalian Cytogenetics (Chromosomal Aberration Assay in Human Peripheral Blood Lymphocytes) OPPTS 870.5375 [§84-2]; OECD 473.

PC CODE: 120301 **DP BARCODE:** D294559
TXR#: 0052299 **SUBMISSION NO.:** None

TEST MATERIAL (PURITY): Thidiazuron (98.7% a.i.)

SYNONYMS: AE B049537; *N*-phenyl-N'-1,2,3-thiadiazol-5-ylurea

CITATION: Gudi, R. and C.M. Brown. (2001) Thidiazuron technical: *In vitro* mammalian chromosome aberration test. BioReliance, Rockville, MD. Laboratory Study No. AA39KC.341.BTL, April 18, 2001. MRID 46121510. Unpublished.

SPONSOR: Aventis CropScience, 2 TW Alexander Dr., Research Triangle Park, NC

EXECUTIVE SUMMARY - In independently performed mammalian cell cytogenetic (chromosome aberration) assays (MRID 46121510), lymphocyte cultures prepared from human peripheral blood were exposed to Thidiazuron (98.7% a.i., Batch/Lot #: CH107623-02) in dimethyl sulfoxide for 4 hours at concentrations of 0, 9.4, 18.75, 37.5, 75, 150, 200, or 250 µg/mL both in the presence and absence of S9-activation, and for 20 hours at concentrations of 0, 4.7, 9.4, 18.75, 37.5, 75, 150, or 200 µg/mL in the absence of S9-activation. Cells were harvested at 20 hours after initiation of treatment.

Thidiazuron was tested up to cytotoxic concentrations (mitotic suppression; 53-60% at 150 µg/mL [±S9] at 4 hours of exposure and 51% at 37.5 µg/mL [-S9] at 20 hours of exposure). No significant increases in aberration frequency were observed at up to 150 µg/mL (±S9) after 4 hours of exposure or at up to 37.5 µg/mL (-S9) after 20 hours of exposure. The positive controls induced the appropriate response. **There was no evidence of chromosome aberration induced over background in the presence or absence of S9-activation at either time point.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* cytogenetic mutagenicity (chromosome aberration) data.

COMPLIANCE - Signed and dated GLP and Quality Assurance statements were provided. A

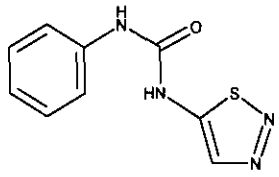
signed Data Confidentiality statement was provided; however, it was not dated.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description: Tan solid
Batch/Lot#: CH107623-02
Purity (w/w): 98.7% a.i.
Stability of compound: Not reported
CAS # of TGAI: 51707-55-2
Structure:



Solvent used: Dimethyl sulfoxide (DMSO)

2. Control materials

Negative control: The solvent alone served as the negative control.
Solvent control: DMSO (100µL/culture)
Positive controls:
Non-activation: Mitomycin C (0.25 and 0.5 µg/mL in distilled water)
Activation: Cyclophosphamide (20 and 40 µg/mL in distilled water)

3. Activation - The S9 fraction was prepared in the laboratory and derived from -

X	Induced	X	Aroclor 1254	X	Rat	X	Liver
	Non-induced		Phenobarbital		Mouse		Lung
			None		Hamster		Other (name)
			Other (name)		Other (name)		

The S9 fraction was stored at ≤70°C until used. The S9 mix consisted of 20 µL S9 fraction, 1 mM NADP, 2 mM $MgCl_2$•6 H_2O, 6 mM KCl, and 1 mM glucose-6-phosphate per milliliter of medium. The final S9 culture concentration was 2%. The S9 fraction was checked for its ability to metabolize 2-aminoanthracene and 7,12-dimethyl-benz(α)anthracene to forms mutagenic to *S. typhimurium* strain TA100.

4. Test cells - Mammalian cells in culture

	Rat lymphocytes		V79 cells (Chinese hamster lung fibroblasts)
	Chinese hamster ovary (CHO) cells	X	Human lymphocytes

Media: Complete medium (RPMI-1640 containing 15% fetal bovine serum , 2 mM L-glutamine, 100 units of penicillin, and 100 µg streptomycin/mL, and supplemented with 1% phytohemagglutinin.

Properly maintained?	X	Yes		No
Periodically checked for *Mycoplasma* contamination? **Not applicable**		Yes		No
Periodically checked for karyotype stability? **Not applicable**		Yes		No

Whole blood was collected from two healthy non-smoking male volunteers (one each for the preliminary and main studies) with no recent history of radiotherapy, viral infection, or the administration of drugs. Whole blood cultures were established by placing 0.6 mL heparinized blood into tubes containing 9.4 mL of complete medium supplemented with 1% phytohemagglutinin. The cultures were incubated at 37±1°C in a humidified 5±1% CO_2 atmosphere for 44-48 hours.

5. Test compound concentrations used

Non-activated
conditions: 0, 9.4, 18.75, 37.5, 75, 150, 200, or 250 µg/mL (4 hours)
(duplicate cultures) 0, 4.7, 9.4, 18.75, 37.5, 75, 150, or 200 µg/mL (20 hours)
(duplicate cultures)

Activated conditions:
(duplicate cultures) 0, 9.4, 18.75, 37.5, 75, 150, 200, or 250 µg/mL (4 hours)

B. TEST PERFORMANCE

1. Preliminary cytotoxicity assay - A preliminary cytotoxicity test was performed to determine mitotic suppression after 4 hours of exposure at concentrations of 0.5, 1.5, 5.0, 15, 50, 150, 500, 1500, or 5000 µg/mL in the presence and absence of S9-activation. Additionally, a 20 hour exposure at the same concentrations was performed in the absence of S9.

2. Cytogenetic assay

a. Cell exposure time	Test Material	Solvent Control	Positive Control
Non-activated:	4 h	4 h	4 h
	20 h	20 h	20 h
Activated:	4 h	4 h	4 h

b. Spindle inhibition

Inhibitor used (concentration): Colcemid® (1.0 µg/mL in deionized water)
Administration time: Approximately 2 hours (before cell harvest)

c. Harvest time after initiation of treatment	Test Material	Solvent Control	Positive Control
Non-activated:	20 h	20 h	20 h
Activated:	20 h	20 h	20 h

d. Details of slide preparation - After cell division was arrested, the cultures were centrifuged, and then cell swelling was induced by treatment with a 0.075M KCl solution for 20 minutes at 37±1°C. The cells were mixed and 0.5 mL of fixative (methanol:glacial acetic acid, 3:1 v/v) was added to each tube. The cultures were centrifuged, the supernatant was discarded, the cells were fixed twice with washes of 3-5 mL of the fixative, and stored at least overnight at 2-8°C. The fixed cells were centrifuged, the supernatant was removed, and the cells were resuspended in 1 mL fresh cold fixative. The cells were centrifuged, and a few drops of the cell suspension were then placed on microscope slides and allowed to air-dry overnight. The cells were then stained with 5% Giemsa, air-dried, and coverslipped. Two slides per culture were prepared. All slides were coded prior to evaluation.

e. Metaphase analysis

No. of cells examined per dose: 200 (100/replicate)

Scored for structural?	[X]	Yes	[ ]	No
Scored for numerical?	[X]	Yes, polyploidy and endoreduplication	[ ]	No
Coded prior to analysis?	[X]	Yes	[ ]	No

f. Evaluation criteria - Only metaphases that contained the appropriate number of chromosomes were scored.

Assay validity - The assay was considered valid if both of the following criteria were met:
- The aberration frequencies of the positive controls were significantly ($p \leq 0.05$) higher than the solvent controls.
- The aberration frequency of the solvent controls were within the historical control range.

Positive result - The assay was considered positive if either of the following criteria was met:
- The aberration frequency was increased in a dose-responsive manner with at least one concentration being significantly ($p \leq 0.05$) higher than the concurrent solvent control.
- A reproducible significant ($p \leq 0.05$) increase in aberration frequency compared to the concurrent solvent controls was observed at any one dose without a dose-response.

g. Statistical analysis - It was stated that Fisher's exact test was used to analyze the incidence of aberrations (%). Statistical significance was determined at $p \leq 0.05$.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY CYTOTOXICITY ASSAY - Neither the pH nor the osmolality of the

medium was affected by addition of the test material. A visible precipitate was noted at concentrations ≥500 µg/mL. Moderate toxicity (mitotic index reduced by ≥50%) was observed at 150 µg/mL in the 4 hour exposures (±S9), and at 50 µg/mL in the 20 hour exposure (-S9). The test material induced complete cytotoxicity at ≥500 µg/mL (4 hours, ±S9) and at ≥150 µg/mL (20 hours, -S9). Based on the results of the preliminary cytotoxicity test, concentrations of 0, 9.4, 18.75, 37.5, 75, 150, 200, or 250 µg/mL (±S9) were chosen for the 4 hour exposure, and concentrations of 0, 4.7, 9.4, 18.75, 37.5, 75, 150, or 200 µg/mL (-S9) were selected for the 20 hour exposure in the definitive mutagenicity assay.

B. CYTOGENETIC ASSAY - The results of the cytogenetic assays were presented in the study report in Tables 4-6 on pages 18-20. As the results of these assays were negative, a copy of summary Table 7 on page 21 of the study report is included as an attachment to this DER. Based on the cytotoxicity observed, concentrations of ≤150 µg/mL were evaluated in the 4 hour groups (±S9), and concentrations of ≤37.5 µg/mL were evaluated in the 20 hour exposure group (-S9). No significant increases in aberration frequency compared to solvent controls were observed at any concentration in the presence or absence of S9 at 4 hours exposure or in the absence of S9 after 20 hours of exposure. The positive controls induced increased ($p \leq 0.01$) aberration frequencies in all trials.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATOR'S CONCLUSIONS - The investigator concluded that thidiazuron did not induce chromosome aberrations in cultured human peripheral blood lymphocytes at up to 150 µg/mL (±S9) after 4 hours of exposure or up to 37.5 µg/mL (-S9) after 20 hours of exposure.

B. REVIEWER COMMENTS - No significant increases in aberration frequency were observed at up to 150 µg/mL (±S9) after 4 hours of exposure or at up to 37.5 µg/mL (-S9) after 20 hours of exposure. The positive controls induced the appropriate response. **There was no evidence of chromosome aberration induced over background in the presence or absence of S9-activation at either time point.**

This study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5375, OECD 473) for *in vitro* cytogenetic mutagenicity (chromosome aberration) data.

C. STUDY DEFICIENCIES - The following deficiency was noted but does not alter the conclusions of this DER:

- The dose formulations were not analyzed for actual concentrations of test substance.

ATTACHMENT

**THE FOLLOWING ATTACHMENT IS NOT AVAILABLE ELECTRONICALLY
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TABLE 7
SUMMARY

Treatment	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored	Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
DMSO	-	4	5.9	200	0.000	±0.000	0.0	0.0
Thidiazuron, Technical								
37.5 ug/mL	-	4	5.5	200	0.000	±0.000	0.0	0.0
75 ug/mL	-	4	5.0	200	0.000	±0.000	0.0	0.0
150 ug/mL	-	4	2.8	200	0.005	±0.071	0.0	0.5
MMC 0.5 ug/mL	-	4	5.3	200	0.120	±0.355	0.0	11.0**
DMSO	+	4	6.7	200	0.000	±0.000	0.0	0.0
Thidiazuron, Technical								
37.5 ug/mL	+	4	5.7	200	0.000	±0.000	0.0	0.0
75 ug/mL	+	4	5.0	200	0.000	±0.000	0.0	0.0
150 ug/mL	+	4	2.7	200	0.000	±0.000	0.0	0.0
CP 20 ug/mL	+	4	5.8	200	0.070	±0.256	0.0	7.0**
Solvent	-	20	7.0	200	0.000	±0.000	0.0	0.0
Thidiazuron, Technical								
9.4 ug/mL	-	20	6.3	200	0.000	±0.000	0.0	0.0
18.75 ug/mL	-	20	5.9	200	0.000	±0.000	0.0	0.0
37.5 ug/mL	-	20	3.4	200	0.000	±0.000	0.0	0.0
MMC 0.25 ug/mL	-	20	5.6	200	0.155	±0.402	0.0	14.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, $p \leq 0.05$; **, $p \leq 0.01$; using the Fisher's exact test.

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Chemical:	**Thidiazuron**
PC Code:	**120301**
HED File Code	**13000 Tox Reviews**
Memo Date:	**12/09/2004**
File ID:	**TX0052299**
Accession Number:	**412-05-0094**

HED Records Reference Center
04/12/2005