UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM* :
October 19, 2005 (original date March 10, 1998)

SUBJECT: **THIDIAZURON:** Review of several developmental toxicity (teratology) study in rats and rabbits.

EPA DP Barcode: D230151; EPA Submission# S511959; EPA MRID No.s 43853001, 00077126, 94246035 (reformat of 00077127), 00077128, 94246036 (reformat of 00077129; EPA Pesticide Chemical Code 120301, Toxicology Chemical Code 659A, Reregistration Case# 4092

TO: Arnold Layne/Bill Wooge, PM 51
SRRD (7508W)

FROM: Stephen C. Dapson, Ph.D.
Branch Senior Scientist
Toxicology Branch II/HED (7509C)

THRU: K. Clark Swentzel
Branch Chief
Toxicology Branch II/HED (7509C)

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

Action Requested: Review a developmental toxicity (teratology) study in rats with Thidiazuron.

Recommendations: TBII has reviewed the study *Thidiazuron: rat oral teratology and range-finding studies*. (AgrEvo UK Limited, Toxicology Function for AgrEvo USA Company, Study Number TOX 94220/94221, October 24, 1995; EPA MRID Number 43853001). Also TBII examined the toxicology database for Thidiazuron and determined that four developmental toxicity (teratology) studies (2 in the rat and 2 in the rabbit) needed to be rereviewed for reregistration purposes (to update the DERs and produce Executive Summaries). The following are the conclusions from these reviews:

*This memorandum was generated and electronically finalized on March 10, 1998. The memorandum and the review of MRID 43853001 were misfiled and were not retrievable. This signed memorandum is identical to the original produced in 1998 and based on the available original electronic file in Macintosh format.

I. Thidiazuron: rat oral teratology and range-finding studies. (AgrEvo UK Limited, Toxicology Function for AgrEvo USA Company, Study Number TOX 94220/94221, October 24, 1995; EPA MRID Number 43853001.

In a developmental toxicity study (MRID# 43853001), 22 pregnant Wistar rats per group were administered Thidiazuron (99% a.i.) by gavage at doses of 0, 4, 38, or 360 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, all dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Individual placental weights and placental abnormalities were recorded. The neck and the thoracic and abdominal cavities of approximately one-half of each litter were dissected and examined, the fetuses were then eviscerated and processed for skeletal examination. The remaining fetuses in each litter were placed in Bouin's fixative for internal examination.

There were no clinical signs of toxicity observed in the controls or low-dose group. Increased salivation was seen in 5/22 ($p < 0.05$) mid-dose animals and 22/22 ($p < 0.01$) high-dose animals. Noisy respiration was also heard in 5/22 ($p < 0.05$) high-dose animals. These signs of toxicity were observed throughout the dosing period in high-dose animals, but only on GD 13-15 in mid-dose animals. One high-dose dam was killed moribund on GD 16 with weight loss, pallor, pale eyes, hunched posture, reduced activity, lowered body temperature, piloerection, irregular respiration, partially closed eyes, yellow staining on the ventral body surface, brown staining around the mouth and nares, and bleeding from the vagina. All other treated and control animals survived to scheduled sacrifice.

There were no treatment-related differences in body weights, food consumption, or water consumption in low- and mid-dose groups as compared with the controls. High-dose dams had significantly ($p \leq 0.05$ or 0.01) lower body weights than the controls beginning on GD 9 and continuing until termination on GD 20. Body weight gains in the high-dose group were 76% of the control group during the dosing interval, but 98% during the post-dosing interval. Throughout the dosing interval, the high-dose group had significantly ($p \leq 0.01$) lower food consumption than controls, but after dosing ended (GD 16-19) there was significantly ($p \leq 0.01$) greater food consumption by the high-dose group as compared to controls. Water consumption by high-dose animals was significantly ($p \leq 0.01$) greater than controls during the treatment interval and continued to be statistically significantly greater than the control level on GD 16-17 after cessation of treatment.

At maternal necropsy on GD 20, hair loss on the ventral body surface was observed in 0/22, 0/22, 0/22, and 5/21 ($p \leq 0.05$)

animals in the 0, 4, 38, and 360 mg/kg/day groups, respectively. One high-dose dam had yellow staining around the urogenital area and a large amount of dark brown fluid in one uterine horn. There were no other compound-related gross lesions in any treated animals.
Therefore, the maternal toxicity LOEL is 38 mg/kg/day based on clinical signs of toxicity (salivation) and the maternal toxicity NOEL is 4 mg/kg/day.

There were no statistically significant differences between treated and control groups for numbers of corpora lutea/dam, implantations/dam, fetuses/litter, or resorptions/dam. A slight increase in resorptions per dam in the high-dose group (1.38 vs. a control value of 1.09) was due to two animals with 6 resorptions each. Male, female, and combined fetal body weights from high-dose dams were significantly ($p \leq 0.01$) less than the controls with the combined weights 89% of the control value.

No treatment-related visceral malformations/variations were observed in any fetus from any litter.

A significantly ($p \leq 0.01$) greater number of high-dose litters contained fetuses with external anomalies as compared to controls; this was due to small fetuses. Overall, 9/22, 5/22, 10/22, and 17/21 litters were affected in the 0, 4, 38, and 360 mg/kg/day groups, respectively. Small fetuses (<2.80 g) were observed in 8/22, 5/22, 7/22, and 16/21 ($p \leq 0.01$) litters, respectively. Small placentae (<0.35 g) were observed in 0/22, 1/22, 5/22, and 8/21 ($p \leq 0.01$) litters, respectively.

The number of litters containing fetuses with skeletal anomalies in the 0, 4, 38, and 360 mg/kg/day groups was 12/22, 10/22, 9/22, and 15/21, respectively. Fetuses from high-dose litters showed a generalized reduction in ossification with significantly ($p \leq 0.05$ or 0.01) greater numbers of litters containing fetuses with incomplete ossification of one or more sternebrae, reduced ossification of the vertebrae, and fewer metacarpals/metatarsals ossified. A significantly ($p \leq 0.01$) greater number of high-dose litters with fetuses having a large anterior fontanelle indicated reduced ossification of the skull. There were increased incidences of incomplete ossification of caudal vertebrae (less than 5 ossified) and metacarpals and/or metatarsals incompletely ossified or unossified at the two highest dose levels (fetal incidences of incomplete ossification of caudal vertebrae: 1/124; 2/133; 4/118 ($p = 0.170$ by Fisher's Exact Test); and 5/93 ($p = 0.053$); of metacarpals and/or metatarsals incompletely ossified or unossified: 1/124; 4/133; 6/118 ($p = 0.052$); 6/93 ($p = 0.0255$) at 0, 4, 38 and 360 mg/kg/day, respectively.

When external and skeletal abnormalities are combined, 15/22, 10/22, 13/22, and 18/21 litters in the 0, 4, 38, and 360 mg/kg/day groups contained affected fetuses.

Therefore, the developmental toxicity LOEL is 38 mg/kg/day based on reduced ossification of caudal vertebrae and metacarpals and/or metatarsals. In addition, the incidence of small placentae was increased. These effects, and reduced fetal body weights and increased numbers of small fetuses, also occurred at 360 mg/kg/day. The developmental toxicity NOEL is 4 mg/kg/day. The developmental toxicity LOEL is the same as the maternal toxicity LOEL.

This study is classified as Acceptable/Guideline and satisfies the requirement for a developmental toxicity study (83-3a) in rats.

II. REPROTOX GmbH (1981): Thidiazuron (SN 49 537) Teratology Study in the rat; Reprotox, Reproduktionstoxikologische Auftragsforschung GmbH for NOR-AM Chemical Company and Schering AG; Study Number 413/A; May 11, 1981; EPA MRID Number 00077126.

In a developmental toxicity (teratology) study (MRID# 00077126), groups of 30 pregnant (29 for vehicle control) Specific Pathogen Free rats of the Wistar Han 78 strain (from Schering AG breeding laboratories, Berlin) received either 0, 100, 300, or 900 mg/kg/day Thidiazuron (98.5% a.i.) by oral gavage in 1% CMC from gestation days 6 through 15, inclusive. The investigators recorded mortality or abnormal condition for all animals along with body weights (gestation days 0, 6, 15, and 19). Dams were sacrificed on day 19 of gestation with examinations consisting of congenital abnormalities, macroscopic pathological changes, in the ovaries -the number of corpora lutea, in the uterus - the number and distribution of live fetuses and of resorptions, individual fetal weights were determined and external fetal abnormalities. Any apparent non-pregnant animals had their uteri stained to look for implantation sites. The live fetuses were examined as above and sexed, two thirds of the litters were examined for skeletal abnormalities after staining using the Alizarin staining techniques, the remaining one third of the litters were fixed in Bouin's fixative for examination for visceral abnormalities using the Wilson technique.

Maternal toxicity was noted at 100 mg/kg/day and above in the form of reduced body weight gain during all measured periods. **The Maternal Toxicity LOEL is less than or equal to 100 mg/kg/day and the Maternal Toxicity NOEL is less than 100 mg/kg/day based on reduced body weight gain.**

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Apparent developmental toxicity was noted in the high dose group as an increase in total resorptions and postimplantation loss and reduced mean fetal weight. External, visceral and skeletal data were not adequate for assessment. **The Tentative Developmental Toxicity LOEL is 900 mg/kg/day and the Developmental Toxicity NOEL is 300 mg/kg/day based on increased resorptions, postimplantation loss and reduced mean fetal weight.**

This study is classified as Unacceptable-Guideline and does not satisfy the guideline requirement for a developmental toxicity (teratology) study (§83-3a) in the rat. This study cannot be upgraded; however a repeat study was conducted at lower doses (MRID# 94246035, reformat of 00077127) which satisfies the data requirement (discussed below).

III. Allen, P.A. et al. (1990): T31/2 THIDIAZURON (SN 49 537) Teratology Study in the rat (Reformatted version of revised final report); Reprotox, Reproduktionstoxikologische Auftragsforschung GmbH for NOR-AM Chemical Company and Schering AG; Study Number 536/A; September 26, 1990 (original study dated May 5, 1981); EPA MRID Number 94246035 (reformat of 00077127)

In a developmental toxicity (teratology) study (MRID# 94246035 reformat of 00077127), groups of 30 pregnant Specific Pathogen Free rats of the Wistar Han 78 strain (from Schering AG breeding laboratories, Berlin) received either 0, 25, 50, 100, or 300 mg/kg/day Thidiazuron (99.4% a.i.) by oral gavage in Myrj 53/NaCl from gestation days 6 through 15, inclusive. The investigators recorded mortality or abnormal condition with all animals along with body weights (gestation days 0, 6, 15, and 19). Dams were sacrificed on day 19 of gestation with examinations consisting of congenital abnormalities, macroscopic pathological changes, in the ovaries - the number of corpora lutea, in the uterus - the number and distribution of live fetuses and of resorptions, individual fetal weights were determined and external fetal abnormalities. Any apparent non-pregnant animals had their uteri stained to look for implantation sites. The live fetuses were examined as above and sexed, two thirds of the litters were examined for skeletal abnormalities after staining using the Alizarin staining techniques, the remaining one third of the litters were fixed in Bouin's fixative for examination for visceral abnormalities using the Wilson technique. This study was originally reported on May 5, 1981.

Maternal toxicity was noted at 100 mg/kg/day and above in the form of reduced body weight gain during the dosing period (gestation days 6-15), the post dosing period (gestation days 15-

19) and for the combined dosing plus post dosing period (gestation days 6-19) and for the entire gestation period (except 100 mg/kg/day). **The Maternal Toxicity LOEL is 100 mg/kg/day and the Maternal Toxicity NOEL is 50 mg/kg/day based on reduced body weight gain.**

Developmental toxicity was noted in the high dose group as lower mean fetal weight compared to the control group and there was a slight increase in the number of fetuses in the high dose group with additional thoracic ribs and an increase in the incidence of retarded ossification of the cranium. **The Developmental Toxicity LOEL is 300 mg/kg/day and the Developmental Toxicity NOEL is 100 mg/kg/day based on reduced mean fetal weight and increased skeletal observations.**

This study is classified as Acceptable-Guideline and satisfies the guideline requirement for a developmental toxicity (teratology) study (§ 83-3a) in the rat.

IV. Irvine, L.F.H. (1980): SN 49 537 Oral Teratogenicity Study in the New Zealand White Rabbit; Hazleton Laboratories Europe Limited for NOR-AM Chemical Company and Schering AG; Study Number 2282-14/2; August 1980; EPA MRID Number 00077128.

In a developmental toxicity (teratology) study (MRID# 00077128), groups of 16 pregnant New Zealand White Rabbits (from Ranch Rabbits Ltd., Crawley Down, Sussex) received either 0, 2.5, 7.5, 25, 75, or 250 mg/kg/day Thidiazuron (≈98-99% a.i.) by oral gavage in 1% methyl cellulose in distilled water from gestation days 6 through 18. The investigators recorded general health and behavior for all animals, body weights and food consumption (gestation days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 28). Dams were sacrificed on day 28 of gestation with examinations at sacrifice consisting of a gross necropsy, examining the ovaries for the number of corpora lutea, and in the uterus - the number and distribution of live fetuses and of resorptions along with individual fetal weights, crown-rump length and sex determinations. The live fetuses were examined for external abnormalities, then dissected and examined for visceral abnormalities, then fixed, stained and examined for skeletal abnormalities. The heads were removed after fixation and the brain examined for abnormalities.

Maternal toxicity was noted at the 250 mg/kg/day dose group as reduced body weight gain compared to the control group during the dosing period (-32% of control) with a rebound following dosing. Further, there was reduced body weight gain in the 250 mg/kg/day when the dosing plus post dosing period (gestation days 6-28; 53% of control) and for the entire gestation period (68% of control).

Food consumption was reduced during the dosing period in the high dose group, this was also noted in the high dose group for the post dosing period (gestation day 18-28) and for the dosing plus post dosing period (gestation days 6-28) and the entire gestation period. Food efficiency was reduced in the high dose group during the dosing period and an increase in food efficiency was noted during the post dosing period (gestation days 18-28). Food efficiency for the dosing plus post dosing period and for the entire gestation period was also reduced in the high dose group. **The Maternal Toxicity LOEL is 250 mg/kg/day and the Maternal Toxicity NOEL is 75 mg/kg/day based on reduced body weight gain and reduced food consumption and food efficiency.**

Developmental toxicity was noted as reduced litter size in the high dose group, also there was an increase in total resorptions (early and late) and an increase in postimplantation loss. **The Developmental Toxicity LOEL is 250 mg/kg/day and the Developmental Toxicity NOEL is 75 mg/kg/day based on reduced litter size, increased resorptions and increased postimplantation loss.**

The study is classified as Unacceptable-Guideline and does not satisfy the guideline requirement for a developmental toxicity (teratology) study (§83-3b) in the rabbit. Due to the inadequacy of the dosing solutions, this study cannot be upgraded; however, the repeat study MRID# 94246036 (reformat of 00077129) satisfies this guideline requirement (discussed below).

V. Irvine, L.F.H. (1990): T33/2 SN 49 537: Oral Teratogenicity Study in the New Zealand White Rabbit; Hazleton Laboratories Europe Limited for NOR-AM Chemical Company and Schering AG; Study Number 2458-14/4; September 1990 (original study date January 1981); EPA MRID Number 94246036 (reformat of 00077129).

In a developmental toxicity (teratology) study (MRID# 94246036 reformat of 00077129), group of 16 pregnant New Zealand White Rabbits (from Morton Commercial Rabbits Ltd., Parsonage Farm, Stansted, Essex) received either 0, 2.5, 7.5, 25, 75, or 250 mg/kg/day Thidiazuron (≈98-99% a.i.) by oral gavage in 1% solution of Myrj 53 in 0.9 % w/v saline from gestation days 6 through 18. The investigators recorded general health and behavior for all animals, body weights and food consumption (gestation days 0, 3, 6, 9, 12, 15, 18, 21, 24, and 28). Dams were sacrificed on day 28 of gestation with examinations at sacrifice consisting of a gross necropsy, examining the ovaries for the number of corpora lutea, and in the uterus - the number and distribution of live fetuses and of resorptions along with individual fetal weights, crown-rump length and sex

determinations. The live fetuses were examined for external abnormalities, then dissected and examined for visceral abnormalities, then fixed, stained and examined for skeletal abnormalities. The heads were removed after fixation and the brain examined for abnormalities. This study was originally reported on January 1981.

Maternal toxicity was noted at the 75 and 250 mg/kg/day dose groups as reduced body weight gain as compared to the control group during the dosing period (85% and 45% of control); however, only the high dose group showed a rebound following dosing and when the dosing plus post dosing period (gestation days 6-28) and the entire gestation period are considered, again only the high dose group gained less weight than the control group (82% and 87% of control for the 2 periods). Food consumption was reduced during the dosing period in the high dose group, this was also noted in the high dose group when the dosing plus post dosing period (gestation days 6-28) and the entire gestation period are considered. Food efficiency was reduced in the 75 and 250 mg/kg/day dose groups during the dosing period with an increase in food efficiency noted in both doses during the post dosing period (gestation days 18-28). **The Maternal Toxicity LOEL is 75 mg/kg/day and the Maternal Toxicity NOEL is 25 mg/kg/day based on reduced body weight gain and reduced food efficiency.**

Developmental toxicity was noted as a slightly reduced litter size in the 250 mg/kg/day dose group, and an increase in total resorptions (late) and an increase in postimplantation loss. **The Developmental Toxicity LOEL is 250 mg/kg/day and the Developmental Toxicity NOEL is 75 mg/kg/day based on reduced litter size, increased resorptions and increased postimplantation loss.**

The study is classified as Acceptable-Guideline and satisfies the guideline requirement for a developmental toxicity (teratology) study (§83-3b) in the rabbit.

I. Toxicology Profile for Thidiazuron (40CFR 158.340)

Technical: Thidiazuron
Use Pattern: food

This compound is a registered active ingredient. The following data are required for technical Thidiazuron. This chemical is on **LIST D** for reregistration.

THIS INFORMATION DOES NOT NECESSARILY REFLECT THE DATA REQUIREMENTS FOR REREGISTRATION.

	Required	Satisfied
§81-1 Acute oral toxicity in rats	Yes	Yes
§81-2 Acute dermal toxicity in rabbits	Yes	Yes
§81-3 Acute inhalation toxicity in rats	Yes	**NO***
§81-4 Primary eye irritation in rabbits	Yes	**NO**
§81-5 Primary dermal irritation in rabbits	Yes	Yes
§81-6 Dermal sensitization - guinea pig	Yes	**NO**
§82-1(a) 90 day feeding study - rat	Yes	No*[1]
§82-1(b) 90 day feeding study - nonrodent	Yes	Yes*
§82-2 21 day dermal - rat	Yes	**NO**
§83-1(a) 2-year feeding - rodent	Yes	Yes
§83-1(b) 1 year feeding - nonrodent	Yes	**NO**
§83-2(a) Carcinogenicity - rat	Yes	**NO**
§83-2(b) Carcinogenicity - mouse	Yes	Yes*
§83-3(a) Teratology - rat	Yes	Yes[2]
§83-3(b) Teratology - rabbit	Yes	Yes[2]
§83-4 Multigeneration reproduction-rat	Yes	**NO***
§84-2(a) Mutagenicity Gene Mutation	Yes	Yes
§84-2(b) Muta - Struct.Chromosome Aberr.	Yes	Yes
§84-4 Muta - Other Genotoxic Effects	Yes	Yes
§85-1 General metabolism - rat	Yes	Yes
§85-2 Dermal Penetration (Absorption)	No	

*** = IBT Data**
[1] = satisfied by 2-year chronic feeding study in the rat
[2] = see review attached

II. Data Gaps

The database for technical Thidiazuron is not complete, additional data on the following studies are required for the technical database:

§81-3 Acute inhalation toxicity in rats
§81-4 Primary eye irritation in rabbits
§81-6 Dermal sensitization - guinea pig
§82-2 21 day dermal - rat
§83-1(b) 1 year feeding - nonrodent
§83-2(a) Carcinogenicity - rat
§83-4 Multigeneration reproduction-rat

III. Actions Being Taken to Obtain Additional Information or Clarification

None at this time.

IV. Reference Dose

The RfD has not been established, this chemical will be presented to the RfD/Peer Review Committee for consideration . **(NOTE: The acceptability of all IBT studies will be discussed by the RfD/Peer Review Committee at this meeting; additional data gaps in the Thidiazuron data base may result.)**

V. Pending Regulatory Actions

None at this time.

VI: Toxicological Issues Pertinent to this Request

Chemical on LIST D for reregistration.

A. New toxicology Data on Thidiazuron

Addressed in this memo.

B. Carcinogenicity

No evidence of carcinogenicity was noted in the mouse carcinogenicity study; the rat study must be repeated at higher doses (MTD not obtained in the submitted study).

DATA EVALUATION REPORT

THIDIAZURON

STUDY TYPE: DEVELOPMENTAL TOXICITY - RAT (83-3a)

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 97-009

RHRoss RCar

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Date: 9-9-97

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Managed by Lockheed Martin Energy Research, Corp., for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

EPA Reviewer: Byron T. Backus, Ph.D. Byron T. Backus Date: 10/19/2005
Toxicology Branch 2/HED (7509C)

EPA Branch Senior Scientist: Stephen C. Dapson, Ph.D. Stephen C. Dapson
Toxicology Branch 2/HED (7509C) Date: 10/19/2005

DATA EVALUATION RECORD*

STUDY TYPE: Developmental Toxicity - Rat
OPPTS 870.3700 [§83-3a]

DP BARCODE: D230151
P.C. CODE: 120301

SUBMISSION CODE: S511998
TOX. CHEM. NO.: 659A

TEST MATERIAL (PURITY): Thidiazuron (99% a.i.)

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

CITATION: Baguley, J.K. (1995) Thidiazuron: rat oral teratology and range-finding studies. AgrEvo UK Limited, Toxicology Function, Chesterford Park, Saffron Walden, Essex CB10 1XL, England. Study Number TOX 94220/94221, October 24, 1995. MRID 438530-01. Unpublished.

SPONSOR: AgrEvo USA Company, Little Falls Centre One, 2711 Centerville Road, Wilmington, DE 19808

EXECUTIVE SUMMARY: In a developmental toxicity study (MRID 43853001), 22 pregnant Wistar rats per group were administered Thidiazuron (99% a.i.; Batch No.: CR 19111/02/940301) by gavage at doses of 0, 4, 38, or 360 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, all dams were sacrificed, subjected to gross necropsy, and all fetuses examined externally. Individual placental weights and placental abnormalities were recorded. The neck and the thoracic and abdominal cavities of approximately one-half of each litter were dissected and examined, the fetuses were then eviscerated and processed for skeletal examination. The remaining fetuses in each litter were placed in Bouin's fixative for internal examination.

There were no clinical signs of toxicity observed in the controls or low-dose group. Increased salivation was seen in 5/22 ($p \leq 0.05$) mid-dose animals and 22/22 ($p \leq 0.01$) high-dose animals. Noisy respiration was also heard in 5/22 ($p \leq 0.05$) high-dose animals. These signs of toxicity were observed throughout the dosing period in the high-dose

*This study was reviewed by the Contractor (Oak Ridge National Laboratories, see cover sheet) and signed on September 9, 1997. The Agency subsequently secondarily reviewed this study and electronically finalized on February 25, 1998. A cover memo was generated for this and other studies (a comparison) and electronically finalized on March 10, 1998. The memo and review were misfiled and were not retrievable. This signed review is identical to the original review produced in 1997/98 based on the available original electronic file in Macintosh format.

animals, but only on GD 13-15 in the mid-dose animals. One high-dose dam was killed moribund on GD 16 with weight loss, pallor, pale eyes, hunched posture, reduced activity, lowered body temperature, piloerection, irregular respiration, partially closed eyes, yellow staining on the ventral body surface, brown staining around the mouth and nares, and bleeding from the vagina. All other treated and control animals survived to scheduled sacrifice.

There were no treatment-related differences in body weights, food consumption, or water consumption between the low- and mid-dose groups as compared with the controls. High-dose dams had significantly ($p \leq 0.05$ or 0.01) lower body weights than the controls beginning on GD 9 and continuing until study termination on GD 20. Body weight gains in the high-dose group were 76% of the control group level during the dosing interval, but 98% during the post-dosing interval indicating recovery. Throughout the treatment interval, the high-dose group had significantly ($p \leq 0.01$) lower food consumption than the controls. Recovery was apparent after treatment ended as indicated by significantly ($p \leq 0.01$) greater food consumption by the high-dose group as compared to the controls during GD 16-19. Water consumption by the high-dose animals was significantly ($p \leq 0.01$) greater than the controls during the treatment interval and continued to be statistically significantly greater than the control level on GD 16-17 after cessation of treatment.

At maternal necropsy on GD 20, hair loss on the ventral body surface was observed in 0/22, 0/22, 0/22, and 5/21 ($p \leq 0.05$) animals in the 0, 4, 38, and 360 mg/kg/day groups, respectively. One high-dose dam had yellow staining around the urogenital area and a large amount of dark brown fluid in one uterine horn. There were no other compound-related gross lesions in any treated animals.

Therefore, the maternal toxicity LOEL is 38 mg/kg/day based on clinical signs of toxicity (salivation) and the maternal toxicity NOEL is 4 mg/kg/day.

There were no statistically significant differences between treated and control groups for numbers of corpora lutea/dam, implantations/dam, fetuses/litter, or resorptions/dam. A slightly increased number of resorptions per dam occurred in the high-dose group, but was due to two animals with 6 resorptions each. Male, female, and the combined fetal body weights from high-dose dams were significantly ($p \leq 0.01$) less than the controls with the combined weights 89% of the control value.

No treatment-related visceral malformations/variations were observed in any fetus from any litter.

A significantly ($p \leq 0.01$) greater number of high-dose litters contained fetuses with external anomalies as compared to controls. Overall, 9/22, 5/22, 10/22, and 17/21 litters were affected in the 0, 4, 38, and 360 mg/kg/day groups, respectively. Small fetuses (<2.80 g) were observed in 8/22, 5/22, 7/22, and 16/21 ($p \leq 0.01$) litters,

respectively. Small placentae (<0.35 g) were observed in 0/22, 1/22, 5/22, and 8/21 ($p \leq 0.01$) litters, respectively.

The number of litters containing fetuses with skeletal anomalies in the 0, 4, 38, and 360 mg/kg/day groups was 12/22, 10/22, 9/22, and 15/21, respectively. Fetuses from high-dose litters showed a generalized reduction in ossification with significantly ($p \leq 0.05$ or 0.01) greater number of litters containing fetuses with incomplete ossification of one or more sternebrae, reduced ossification of the vertebrae, and fewer metacarpals/metatarsals ossified. A significantly ($p \leq 0.01$) greater number of high-dose litters with fetuses having a large anterior fontanelle indicated reduced ossification of the skull.

When external and skeletal abnormalities are combined, 15/22, 10/22, 13/22, and 18/21 litters in the 0, 4, 38, and 360 mg/kg/day groups contained affected fetuses.

Therefore, the developmental toxicity LOEL is 38 mg/kg/day based on reduced ossification of caudal vertebrae and metacarpals and/or metatarsals. In addition, the incidence of small placentae was increased. These effects, and reduced fetal body weights and increased numbers of small fetuses, also occurred at 360 mg/kg/day. The developmental toxicity NOEL is 4 mg/kg/day. The developmental toxicity LOEL is the same as the maternal toxicity LOEL.

This study is classified as Acceptable/Guideline and satisfies the requirement for a developmental toxicity study (83-3a) in rats.

COMPLIANCE: Signed and dated Quality Assurance, Good Laboratory Practice Statements, Data confidentially and Flagging statements were included.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Thidiazuron

Description: beige colored powder
Batch No.: CR 19111/02/940301
Purity: 99% a.i.
Stability of compound: reanalysis at end of study confirmed initial purity
CAS No.: 51707-55-2
Structure:

2. Vehicle an/d/or positive control

A 1% carboxymethylcellulose solution was used as the vehicle. No positive control was used.

3. Test animals

Species: Rat
Strain: HanIbm Wistar
Age and weight at study initiation: 10-11 weeks; 170-209 g
Source: Biological Research Laboratories Limited, Wölfer Strasse 4, CH-4414 Füllinsdorf, Basle, Switzerland
Housing: Females were housed individually in cages consisting of high density polypropylene bodies with lids and floors of stainless steel grid. Cages were suspended over trays covered with absorbent crêpe paper.
Diet: SDS LAD1 SQC expanded pellet laboratory animal diet supplied by Special Diets Services, Limited, Witham, Essex, England was available *ad libitum*. Tap water was available *ad libitum*.
Environmental conditions:
Temperature: 19-25°C
Humidity: 40-70%
Air changes: 15/hour
Photoperiod: 12 hour light/12 hour dark
Acclimation period: 7 days

B. PROCEDURES AND STUDY DESIGN

This study was designed to assess the developmental toxicity potential of Technical Thidiazuron when administered by gavage to Wistar rats on gestation days 6 through 15, inclusive.

1. In life dates

Start: July 5, 1994; end: August 4, 1994

2. Mating

Females were paired 1:1 with stock males of the same strain. Each morning, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which a sperm positive vaginal smear or at least three copulation plugs were found was designated as Day 0 of gestation.

3. Animal assignment and dose selection are presented in Table 1. Animals showing unequivocal evidence of mating were allocated to group and cage position in sequence, thus ensuring that animals mated on any one day were evenly distributed between the groups.

TABLE 1: Animal assignment

Group	Dose (mg/kg/day)	Number assigned
Control	0	22
Low dose	4	22
Mid dose	38	22
High dose	360	22

Data taken from p. 21, MRID 43853001.

4. Dose selection rationale

Doses were selected based on a range-finding study of Thidiazuron in pregnant Wistar rats. The results of this study were included with the main study and are summarized in the Appendix. Briefly, groups of six pregnant Wistar rats were given 0, 100, 500, or 1000 mg Thidiazuron/kg/day by gavage on GD 6-15. Excessive maternal toxicity was observed at the high dose and 4 animals were killed *in extremis*. At ≥500 mg/kg/day, animals had decreased maternal weight gain and food consumption, increased maternal water consumption, increased fetal resorptions, and decreased fetal body weights. No effects were observed at 100 mg/kg/day. Therefore, doses for the main study were chosen as 0, 4, 38, and 360 mg/kg/day.

5. Dosing

All doses were in a volume of 10 mL/kg of body weight/day prepared the day before administration. Dosing was based on the animal's body weight on that day.

6. Dose solution preparation and analysis

Dosing solutions were prepared the day before administration as a suspension in 1% carboxymethylcellulose. Dosages were calculated on the basis of the test material as supplied.

The dosing solutions were analyzed by the Sponsor for concentration, homogeneity and stability. Samples were taken on dosing days 1, 8, and 12 immediately after preparation, after resuspension, prior to dosing, after dosing approximately one-half of the animals, and immediately after dosing all animals in the group. The results of the analyses were not included with the study report, however, the author stated that all analyzed concentrations were 90.6 to 100% of nominal.

C. OBSERVATIONS

1. Maternal observations and evaluations

The animals were checked daily for clinical signs of toxicity. Maternal body weights were determined on GD 0, 3, 6-16, 18, and 20. Food and water consumption were measured for the intervals of GD 0-2, 3-5, 6-8, 9-11, 12-15, 16-17, and 18-19. Dams were sacrificed on GD 20 by carbon dioxide inhalation and examined grossly. The reproductive tract of each animal, including ovaries, was removed and examined for number of corpora lutea, number of implantation sites, number of resorptions, and number and distribution of live and dead fetuses.

2. Fetal evaluations

Each fetus was weighed, sexed, and examined for external abnormalities. Individual placental weights and placental abnormalities were recorded. The neck and the thoracic and abdominal cavities of approximately one-half of each litter were dissected and examined, the fetuses were then eviscerated and processed for skeletal examination. The remaining fetuses in each litter were placed in Bouin's fixative for internal examination.

D. DATA ANALYSIS

1. Statistical analysis

Methods of statistical analyses were described as footnotes to the data summary tables. Maternal body weights, food consumption, and water consumption were analyzed using a one-way Analysis of Variance (ANOVA) followed by a t-test to separate means. Fetal body weights were analyzed with a weighed t-test following a nested one-way ANOVA. Differences associated with a probability of $p < 0.05$ were considered to be statistically significant. The method of evaluation of placental weights and incidence data was not described in either the text or tables suggesting that these data were not analyzed statistically.

2. Historical control data were provided to allow comparison with concurrent controls.

*This study was reviewed by the Contractor (Oak Ridge National Laboratories, see cover sheet) and signed on September 9, 1997. The Agency subsequently secondarily reviewed this study and electronically finalized on February 25, 1998. A cover memo was generated for this and other studies (a comparison) and electronically finalized on March 10, 1998. The memo and review were misfiled and were not retrievable. This signed review is identical to the original review produced in 1997/98 based on the available original electronic file in Macintosh format.

II. RESULTS

A. MATERNAL TOXICITY

1. Mortality and clinical signs

Clinical signs of toxicity were observed after dosing in the mid- and high-dose groups. Increased salivation was seen in 5/22 ($p \leq 0.05$) mid-dose animals and 22/22 ($p \leq 0.01$) high-dose animals. Noisy respiration was also heard in 5/22 ($p \leq 0.05$) high-dose animals. These signs of toxicity were observed throughout the dosing period in the high-dose animals, but only on GD 13-15 in the mid-dose animals. The controls and low-dose animals were unaffected.

One high-dose dam was killed moribund on GD 16 with weight loss, pallor, pale eyes, hunched posture, reduced activity, lowered body temperature, piloerection, irregular respiration, partially closed eyes, yellow staining on the ventral body surface, brown staining around the mouth and nares, and bleeding from the vagina. All other treated and control animals survived to scheduled sacrifice.

2. Body weight

Selected maternal body weight data are given in Table 2. High-dose dams had significantly ($p \leq 0.05$ or 0.01) lower body weights than the controls beginning on GD 9 and continuing until study termination on GD 20. Body weight gains in the high-dose group were 76% of the control group level during the dosing interval, but 98% during the post-dosing interval indicating recovery. There were no other treatment-related differences in body weights between the treated and control groups.

TABLE 2: Maternal body weights and weight gains during gestation (g)				
GD	0 mg/kg/day	4 mg/kg/day	38 mg/kg/day	360 mg/kg/day
0	191 ± 5	192 ± 8	191 ± 7	190 ± 8
6	217 ± 8	218 ± 9	216 ± 8	217 ± 11
9	227 ± 9	231 ± 10*	226 ± 9	225 ± 12*
12	244 ± 10	248 ± 13	243 ± 10	235 ± 13**
16	271 ± 11	277 ± 14*	268 ± 13	258 ± 17**
20	320 ± 17	327 ± 19	317 ± 17	306 ± 21**
Weight gain GD 6-16[a]	54	59	52	41 (76)[b]
Weight gain GD 16-20[a]	49	50	49	48 (98)
Weight gain GD 0-20[a]	129	135	126	116 (90)

Data taken from Table 2, p. 33, MRID 43853001.
[a]Calculated by reviewer.
[b]Number in parentheses is per cent of control.
Significantly different from control: *$p \leq 0.05$, **$p \leq 0.01$.

3. Food and water consumption

Food and water consumption data are summarized in Table 3. There were no statistically significant differences in food or water consumption between the low- and mid-dose groups as compared to the control group. During the treatment interval, the high-dose group had significantly ($p \leq 0.01$) lower food consumption than the controls. Recovery was apparent after treatment ended as indicated by the significantly ($p \leq 0.01$) greater food consumption by the high-dose group as compared to the controls during GD 16-19. Water consumption by the high-dose animals was significantly ($p \leq 0.01$) greater than the controls during the treatment interval and continued to be statistically significantly greater than the control level on GD 16-17 after cessation of treatment. Slight recovery had occurred by GD 18-19.

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TABLE 3: Food and water consumption during gestation				
GD	0 mg/kg/day	4 mg/kg/day	38 mg/kg/day	360 mg/kg/day
Food consumption (g/rat/day)				
0-2	18 ± 2	19 ± 2	17 ± 3	18 ± 4
6-8	22 ± 2	24 ± 2	22 ± 2	18 ± 2**
9-11	24 ± 3	24 ± 2	22 ± 2	16 ± 4**
12-15	24 ± 2	25 ± 3	24 ± 2	20 ± 3**
18-19	26 ± 2	27 ± 2	26 ± 2	29 ± 2**
Water consumption (mL/rat/day)				
0-2	35 ± 13	35 ± 10	35 ± 6	34 ± 13
6-8	38 ± 10	44 ± 11	42 ± 11	62 ± 24**
9-11	38 ± 8	45 ± 15	42 ± 10	67 ± 32**
12-15	42 ± 8	50 ± 19	49 ± 11	80 ± 27**
18-19	41 ± 12	58 ± 48	47 ± 16	56 ± 13

Data taken from Tables 3 and 4, pp. 34 and 35, respectively, MRID 43853001.
**Significantly different from control, $p \leq 0.01$.

4. Gross pathology

At maternal necropsy on GD 20, hair loss on the ventral body surface was observed in 0/22, 0/22, 0/22, and 5/21 ($p \leq 0.05$) animals in the 0, 4, 38, and 360 mg/kg/day groups, respectively. One high-dose dam had yellow staining around the urogenital area and a large amount of dark brown fluid in one uterine horn. There were no other compound-related gross lesions in any treated animals.

The one high-dose dam killed moribund on GD 16 had red thymic and mesenteric lymph nodes, dark areas on the lungs, pale liver and kidneys, pale and thickened spleen, reduced and dehydrated cecal contents, and numerous lesions in the stomach and small intestine.

5. Cesarean section data

Data collected at cesarean section are summarized in Table 4. There were no statistically significant differences between treated and control groups for numbers of corpora lutea/dam, implantations/dam, fetuses/litter, or resorptions/dam. The slightly greater number of resorptions per dam in the high-dose group was due to two animals with 6 resorptions each. Male, female, and the combined fetal body weights from high-dose dams were significantly ($p \leq 0.01$)

less than the controls with the combined weights 89% of the control value.

The one high-dose dam killed moribund on GD 16 had 14 corpora lutea, 13 implantations with 3 late resorptions, and 10 viable fetuses. Clotted blood was observed around the amniotic sacs and placentae of 2 implantations. This animal has been excluded from the group means and totals in Table 4 except for total pregnant and pregnancy rate.

TABLE 4. Cesarean section observations

Observation	0 mg/kg/day	4 mg/kg/day	38 mg/kg/day	360 mg/kg/day
No. animals assigned	22	22	22	22
No. animals pregnant	22	22	22	22
Pregnancy rate (%)	100	100	100	100
Maternal mortality	0	0	0	1
Delivered early/aborted	0	0	0	0
Corpora lutea/Dam	12.7 ± 1.3	13.0 ± 1.3	12.3 ± 2.1	12.4 ± 1.2
Implantation/Dam	11.9 ± 1.6	12.1 ± 2.3	11.2 ± 3.0	11.6 ± 2.2
Preimplantation loss (%)	6.8	7.0	8.9	7.3
Postimplantation loss (%)	9.2	6.0	6.5	11.9
Total live fetuses	238	250	230	214
Live fetuses/litter	10.8 ± 1.9	11.4 ± 2.2	10.5 ± 2.9	10.2 ± 2.9
Mean fetal weight (combined) (g)	3.39 ± 0.07	3.40 ± 0.07	3.34 ± 0.08	3.02 ± 0.08**
Male	3.46 ± 0.11	3.50 ± 0.09	3.45 ± 0.11	3.10 ± 0.14**
Female	3.31 ± 0.09	3.30 ± 0.09	3.26 ± 0.11	2.98 ± 0.09**
Sex ratio (% Male)	55	53	50	42
Total resorptions[a]	24	16	16	29
Resorptions/Dam	1.09 ± 1.04	0.73 ± 0.85	0.73 ± 0.85	1.38 ± 1.18
Early resorptions	1.09 ± 1.04	0.73 ± 0.85	0.73 ± 0.85	1.38 ± 1.18
Late resorptions	0.0	0.0	0.0	0.0
Dams with total litter resorption	0	0	0	0
Placental weight (g)	0.48 ± 0.02	0.49 ± 0.02	0.48 ± 0.03	0.51 ± 0.03[b]

Data taken from Tables 6-8 and Appendix 7, pp. 37-39 and 68, respectively, MRID 43853001.
[a]Calculated by reviewer.
[b]Significantly different from control, $p \leq 0.05$; calculated by reviewer using ANOVA followed by Dunnett's test.
**Significantly different from control, $p \leq 0.01$.

B. DEVELOPMENTAL TOXICITY

No treatment-related visceral malformations/variations were observed in any fetus from any litter. When external and skeletal abnormalities are combined, 15/22, 10/22, 13/22, and 18/21 litters in the 0, 4, 38, and 360 mg/kg/day groups contained affected fetuses. The main observations were of small fetuses, small placentae, and reduced ossification.

1. External examination

External malformations/variations are given in Table 5a. A significantly ($p \leq 0.01$) greater number of high-dose litters contained fetuses with external anomalies as compared to controls. Overall, 9/22, 5/22, 10/22, and 17/21 litters were affected in the 0, 4, 38, and 360 mg/kg/day groups, respectively. The most common abnormalities in fetuses from high-dose litters were small fetuses and small placentae.

2. Visceral examination

There were no dose- or treatment-related visceral malformations/variations. Common findings in both treated and control litters included hepatic hemorrhages, small additional liver lobe, testes slightly displaced, and subcutaneous hemorrhages. However, the incidence rate for each of these findings was within the historical control range.

3. Skeletal examination

Skeletal malformations/variations are given in Table 5b. Because the individual animal data were summarized by litter and not fetus and because some endpoints (i.e., incomplete ossification of sternebrae) were listed separately, it was not possible to determine the total number of fetuses affected with skeletal anomalies. The number of litters containing fetuses with skeletal anomalies in the 0, 4, 38, and 360 mg/kg/day groups was 12/22, 10/22, 9/22, and 15/21, respectively. Fetuses from high-dose litters showed a generalized reduction in ossification with significantly ($p \leq 0.05$ or 0.01) greater number of litters containing fetuses with incomplete ossification of one or more sternebrae, reduced ossification of the vertebrae, and fewer metacarpals/metatarsals ossified. The significantly ($p \leq 0.01$) greater number of high-dose litters with fetuses having a large anterior fontanelle suggests reduced ossification of the skull.

TABLE 5a. External malformations/variations

Observation	0 mg/kg/day	4 mg/kg/day	38 mg/kg/day	360 mg/kg/day
No. Pups (litters) examined	238 (22)	250 (22)	230 (22)	214 (21)
No. Pups (litters) affected	13 (9)	6 (5)	20 (10)	49 (17)**
Small fetus (<2.80 g)	11 (8)	5 (5)	12 (7)	45 (16)**
Small placenta (<0.35 g)	0 (0)	1 (1)	10 (5)	10 (8)**
Domed head	2 (2)	1 (1)	0 (0)	3 (2)

Data taken from Table 8 and Appendix 7, pp. 39 and 68-71, respectively, MRID 43853001.
**Litter incidence significantly different from control, $p \leq 0.01$; calculated by reviewer using Fisher's Exact test.

TABLE 5b. Skeletal malformations/variations

Observation	0 mg/kg/day	4 mg/kg/day	38 mg/kg/day	360 mg/kg/day
No. Pups (litters) examined	124 (22)	133 (22)	118 (22)	112 (21)
No. Litters affected	12[a]	10	9	15
Large anterior fontanelle	5 (3)	5 (4)	9 (6)	19 (11)**
Incomplete ossification of 1 sternebra	57 (19)	46 (19)	32 (17)	18 (12)*
Incomplete ossification of 3 sternebrae	10 (8)	11 (8)	13 (9)	29 (16)**
Incomplete ossification of 6 sternebrae	0 (0)	1 (1)	0 (0)	4 (4)*
Ossification of ventral arch of 1st cervical vertebra	7 (7)	7 (4)	2 (2)	1 (1)*
Incomplete ossification of caudal vertebrae (<5 ossified)	1 (1)	2 (2)	4 (3)	5 (5)
Metacarpals/metatarsals 4/4	50 (19)	57 (19)	51 (17)	23 (12)*
Metacarpals and/or metatarsals incompletely ossified or unossified	1 (1)	4 (4)	6 (4)	6 (6)*

Data taken from Table 9 and Appendix 9, pp. 41-45 and 76-80, respectively, MRID 43853001.
[a]It was not possible to determine the number of fetuses affected.
Litter incidence significantly different from control, *$p \leq 0.05$, **$p \leq 0.01$; calculated by reviewer using Fisher's Exact Test.

III. DISCUSSION

A. INVESTIGATORS' CONCLUSIONS

The study author concluded that administration of Thidiazuron to Wistar rats on GD 6-15 resulted in maternal and fetal toxicity at a dose of 360 mg/kg/day. At this dose, dams showed poor condition, low weight gain, low food consumption, and high water consumption, and exhibited recovery after the treatment interval. Fetal body weights were low, and many fetuses showed developmental delay, including reduced skeletal ossification. Findings at 38 mg/kg/day (LOEL) were restricted to a slight fetal immaturity, indicated by a slight reduction in sternebrae and metacarpal/metatarsal ossification. Therefore, the study author chose 4 mg/kg/day as the maternal and fetal NOEL.

B. REVIEWER'S DISCUSSION

1. MATERNAL TOXICITY

Clear evidence of maternal toxicity was observed at 38 and 360 mg/kg/day. Post-dosing salivation was seen in mid-dose animals and salivation and noisy respiration were reported for high-dose animals. Because the salivation was transient and did not appear until the end of the dosing interval in the mid-dose animals, the study author concluded that this clinical sign was of no toxicological significance. However, due to the dose-response relationship in the number of animals affected and the appearance of salivation following dosing, the reviewer feels that this is clearly due to treatment.

More pronounced maternal toxicity occurred at the high dose. Dams in this group had reduced body weights that correlated with lower food consumption, increased water consumption, and hair loss on the ventral body surface. The sacrifice of one high-dose dam on GD 16 is considered treatment-related.

2. DEVELOPMENTAL TOXICITY

a. Deaths/resorptions

At maternal necropsy on GD 20, no dead fetuses were reported. The slightly greater number of resorptions per dam in the high-dose group was due to two animals with 6 resorptions each. Clinical signs in these dams were limited to post-dosing salivation suggesting that the increase in resorptions in these animals was not due to excessive maternal toxicity.

b. Altered growth

In high-dose litters, delayed fetal maturation was evident in the significantly lower body weights and reduced ossification. These types of anomalies are common sequelae to maternal toxicity. [Note added by EPA reviewer]: There were increased incidences of incomplete ossification of caudal vertebrae (less than 5 ossified) and metacarpals and/or metatarsals incompletely ossified or unossified at the two highest dose levels (fetal incidences of incomplete ossification of caudal vertebrae: 1/124; 2/133; 4/118 ($p = 0.170$ by Fisher's Exact Test); and 5/93 ($p = 0.053$); of metacarpals and/or metatarsals incompletely ossified or unossified: 1/124; 4/133; 6/118 ($p = 0.052$); 6/93 ($p = 0.0255$) at 0, 4, 38 and 360 mg/kg/day, respectively. The findings at 38 mg/kg/day are part of dose-related trends and sufficiently near statistical significance so as to be considered indications of fetal toxicity (although, as stated by the contract reviewer, they are probably secondary to maternal toxicity).

c. Developmental variations

Fetuses in litters from high-dose dams had significantly reduced ossification mainly of the skull, sternebrae, vertebrae, and digits. Reductions in ossification combined with the lower fetal body weights indicate an overall developmental delay.

d. Malformations

There were no dose- or treatment-related malformations observed in any fetuses from any litters.

C. STUDY DEFICIENCIES

Individual fetal data (weights, observations, etc.) were not included, but instead litter summaries were given as the individual data. Therefore, it was not possible to calculate the total number of fetuses affected.

D. CORE CLASSIFICATION

This study is classified as Acceptable/Guideline and satisfies the requirements for a developmental toxicity study (83-3a) in rats.

1. Maternal NOEL = 4 mg/kg/day
2. Maternal LOEL = 38 mg/kg/day based on clinical signs of toxicity (post-dosing salivation)
3. Developmental toxicity NOEL = 4 mg/kg/day

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4. Developmental toxicity LOEL = 28 mg/kg/day based on delayed fetal maturation as indicated by reduced ossification, with reduced fetal weights at the next dose level (360 mg/kg/day).

APPENDIX

Thidiazuron: Rat oral teratology range-finding study

The aim of this study was to select dose levels for the main developmental toxicity study designed to assess the effects of oral administration of Thidiazuran during pregnancy in the rat.

Methods: Groups of six Wistar rats were administered 0, 100, 500, or 1000 mg/kg/day on GD 6-15, inclusive. Thidiazuran (Batch No. CR 19111/02/940301) was administered by gavage as a suspension in 1% carboxymethylcellulose. The source of animals, caging, food and water supplies, mating procedures, and allocation to treatment groups were as described in the main study. Maternal observations, food and water consumption, and body weight data were obtained similar to the main study. On GD 20, females were killed by carbon dioxide inhalation and the reproductive tracts examined for number of corpora lutea, number of implantation sites, number of resorptions, and number and distribution of fetuses. Individual placental weights were determined. Each fetus was weighed, sexed, and examined for external abnormalities. Statistical analyses were not performed on group means.

Results:

A. Maternal toxicity

1000 mg/kg/day - Transient signs of toxicity following dosing included occasional increased salivation or dark eyes. The general condition of the animals deteriorated progressively with signs of poor grooming, piloerection, hunched posture, ptosis, reduced activity, slow/deep/irregular respiration, and reddened skin color. One female was killed moribund on GD 11 and three were killed moribund on GD 15. Due to the deterioration in health of the animals in this group, dosing was discontinued after 6-9 doses. The general condition of the surviving animals showed improvement after cessation of dosing. Body weights at the end of the treatment period were 74% of the control level. Food and water consumption during the treatment interval were 27-54% and 100-200%, respectively, of the control amounts. Gross necropsy of premature decedents revealed abnormal contents of the gastrointestinal tract, mottled spleens, pale kidneys, a hemorrhagic urinary bladder, large, red, mandibular lymph nodes, and/or blood in the vagina with a large proportion of resorptions.

500 mg/kg/day - Salivation was occasionally observed in a few animals after dosing. Other signs that were observed towards the end of the dosing interval included piloerection, hunched posture, and reddened skin color. One animal was killed moribund on GD 15; necropsy revealed complete litter resorption and abnormal contents of the gastrointestinal tract. Body weights at the end of the treatment period were 86% of the control level. Food and water consumption during the treatment interval were 42-68% and 105-153%, respectively, of the control amounts. At maternal necropsy, one animal had an enlarged spleen.

100 mg/kg/day - Maternal toxicity NOEL

B. Developmental toxicity

1000 mg/kg/day - Both surviving animals had complete litter resorption and one had clear fluid in the uterus.

500 mg/kg/day - There was a slightly increased number of resorptions/litter (mainly due to one female having a 33% post-implantation loss) with a concurrent decrease in fetuses/litter as compared to controls. Fetal and placental weights were 82% and 79%, respectively, of the control values. The incidence rate for small fetuses (<2.80 g) was 47.1% vs. 4% in the controls and for small placenta (<0.35 g) was 25.5% vs. 0% in the controls. Two litters showed thickening of the placentae.

100 mg/kg/day - Developmental toxicity NOEL

Conclusions: The marked maternal toxicity and the associated effects on placental development at 500 and 1000 mg/kg/day were considered to have resulted in immaturity and death of the fetuses rather than a direct embryo/fetal effect of Thidiazuron. Therefore, the study author concluded that a dose between 100 and 500 mg/kg/day would be suitable for use as the high dose in the main developmental toxicity study.