US EPA ARCHIVE DOCUMENT

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

TOXR - 001839
& 002308

APR 30 1982

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

TO: Jay Ellenberger (12)
Registration Division (TS-769)

THRU: Orville E. Paynter, Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: PP#1F2527, 1H5308 and 2139-REE; Request for Tolerances of Thidiazuron on Cotton (0.4 ppm); Milk (0.05 ppm); Eggs (0.1 ppm); Meat, Fat and Byproducts of Cattle, Goats, Hogs, Horses, Poultry and Sheep (0.2 ppm) and Cottonseed Hulls (0.8 ppm), Acc. Nos.: 070127, 099842, 070128, 070129, Tox. Chem. No. ~~274~~ 659A

Registrant: Nor-Am Agricultural Products, Inc.
350 West Shuman Blvd.
Naperville, Illinois 60540

Action Requested:

Establishment of permanent tolerances for cottonseed (0.4 ppm), cottonseed hulls (0.8 ppm), milk (0.05 ppm), eggs (0.1 ppm), meat, fat and byproducts of cattle, goats, hogs, horses, poultry and sheep (0.2 ppm) for residues of the cotton defoliant thidiazuron (N-phenyl-N'-1 2,3-thiadiazol-5-ylurea) and its aniline containing metabolites.

Recommendation:

Toxicology Branch cannot recommend for the establishment of the requested permanent tolerances due to the following major data gaps:

1. Chronic feeding study in the rat
2. One-year feeding study in the dog

The chronic rat study conducted at IBT has been classified as "Supplementary Data" and, due to deficiencies in histopathology, is not adequate to establish a NOEL.

Hence, no chronic toxicity studies are available on which to base the ADI for thidiazuron in support of the proposed tolerances.

It is also recommended that an in vitro cytogenetic test be conducted in a mammalian system.

On November 24, 1981, a Federal Register notice was published establishing temporary tolerances for thidiazuron. The temporary tolerance levels are identical to the requested permanent tolerances and the percentage of the ADI utilized is also identical, i.e., 30.25%.

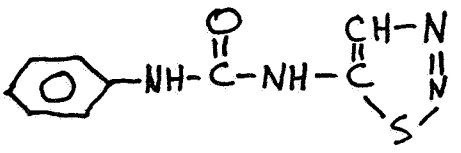
Discussion:

As noted above in the Recommendation section, major data gaps are a chronic feeding/oncogenicity study in the rat and a one year feeding study in the dog. Information regarding subchronic nonrodent toxicity is available from the 90 day dog study (see page 15 of this review), however a dog study of one year duration is normally required for establishment of the proposed tolerances. Limited information regarding chronic toxicity is available from the chronic rat feeding study conducted at IBT (classified as "Supplementary Data", see p. 13 of this review). The chronic rat study is not appropriate for the establishment of an ADI due to deficiencies in histopathology and, given the absence of a one year dog study, a valid study assessing chronic toxicity is not available for this compound.

The provisional ADI (PADI) is currently based on a No Observed Effect Level of 300 ppm in a 90 day dog study. With a 2000 fold safety factor, the PADI for humans is 0.0038 mg/kg bw/day. The requested tolerances would contribute 0.0681 mg/day/1.5 kg of diet for a 60 kg person, thus 30.25% of the PADI will be utilized by the requested tolerances.

2

Chemical Structure:



Toxicology Data Summary:

From the review of 9/7/76 by Reto Engler:

Study	Formulation	Results
Acute Oral, Rat	Tech.	LD_{50} = > 4 g/kg
Acute Oral, Mouse	50W	LD_{50} = 3.9 (Males), 2.6 (Females) g/kg
Acute Oral, Mouse	Tech.	LD_{50} = 5 g/kg
Acute Dermal, Rabbit	Tech.	LD_{50} = 1 g/kg
Acute Dermal, Rabbit	50W	LD_{50} = 1 g/kg
Acute Inhalation, Rat	Tech.	LC_{50} = 2.3 mg/L
Acute Inhalation, Rat	50W	LC_{50} = .25 mg/L
Primary Eye Irritation, Rabbit	Tech.	Unacceptable
Primary Skin Irritation, rabbit	Tech.	Tox. Cat. IV
Primary Skin Irritation, Rabbit	50W	Tox. Cat. IV

From the review of 11/15/78 by Larry Anderson:

Rat Metabolism - Radiolabeled compound is approximately 90% excreted after 96 hours. Radioactivity equally distributed between urine and feces with parent compound primarily in the feces and N-4-hydroxyphenyl-N'-1,2,3-thiadiazol-5-ylurea and phenylurea in the urine.

From the review of 9/23/81 by William Dykstra:

3-Generation Reproduction, Rat	Tech.	NOEL = 200 ppm Based on reduced b.w. during 2nd generation	Core-Minimum IBT Valid

3

From this review:

Ames Assay	Tech.	Negative (both with and without S-9 activation)	Acceptable
Micronucleus, Mouse	Tech.	Negative	Acceptable
Teratology, Rat	Tech.	Inconclusive for fetotoxicity and teratogenic potential.	Supplementary Data
Teratology, Rat	Tech.	NOEL = 100 mg/kg for fetotoxicity. No teratogenic effects observed at HDT.	Core-Minimum
Teratology, Rabbit	Tech.	Inconclusive for fetotoxicity.	Supplementary Data
Teratology, Rabbit	Tech.	NOEL = 25 mg/kg for fetal and maternal toxicity. No teratogenic effects observed at HDT.	Core-Minimum
Chronic Oral, Rat (IBT)	Tech.	Increased mortality in both sexes at 500 ppm, no observed effects at 120 ppm. Inadequate histopathology.	IBT Supplementary Core Supplementary
Oncogenic, Mouse (IBT)	Tech.	No oncogenic potential indicated at doses as high as 1000 ppm.	IBT Valid Core-Minimum
Ninety-Day, Rat (IBT)	Tech.	NOEL = 200 ppm based on increased female gonad weight at 600 ppm.	IBT Valid Core-Minimum
Ninety-Day, Dog (IBT)	Tech.	NOEL = 300 ppm based on decreased b.w. gain in high dose males.	IBT Valid Core-Minimum

Review of Data:

1. Ames Assay. Performed by Inveresk Research International (IRI Project No. 407836), Edinburgh, Scotland and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois.
Submitted on May 29, 1981.

Thidiazuron technical (SN49537, Batch No. 251201 B00000 from Schering AG, Berlin, purity not reported) was dissolved in DMSO and 0.2 ml of solution was added to each tube containing 2 ml soft agar and 0.1 ml bacteria. Amounts of test compound added to each plate were 5 ug, 25 ug, 125 ug, 500 ug, and 2.5 mg. Compound was incubated both with and without S-9 fraction derived from the polychlorinated biphenyl-induced livers of male mice following the general procedures of Ames et al (1975). 2-aminoanthracene was used as a positive control substance and 0.5 ug/plate were added. Strains of TA1535, 100, 1537, 1538 and 98 of S. typhimurium were used.

Results:

A positive response, defined as a doubling of the number colonies observed in control animals, was not observed at levels of test compound between 5 and 500 ug either with or without metabolic activation. At the highest level tested, cytotoxicity was observed and the number of colonies was not reported for any of the 5 strains. A positive response was observed for each of the strains incubated with S-9 fraction in the presence of 2-aminoanthracene. A positive control substance not requiring metabolic activation was not used in this assay.

The results of this assay can be interpreted as demonstrating a lack of mutagenic effect of thidiazuron in the Ames assay.

Classification: "Acceptable"

5

2. Modified Micronucleus Test. Conducted By Schering AG, Berlin, West Germany and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois on May 29, 1981.

a. Preliminary Test Phase I

Thidiazuron technical (97.3% pure) was administered i.p. to Specific Pathogen Free COBS CD-1 (1CR) BR (ICR derived) mice. Test material was dissolved in 1% carboxymethylcellulose and administered at concentrations of 0, 13, 32, 80, 200 and 500 mg/ml (corresponding to 0, 130, 320, 800, 2000 and 5000 mg/kg) to 2 animals/sex/dose level. Animals were observed for 7 days after administration of test compound.

Results:

At 5000 mg/kg, all animals died within 48 hours. At dosages less than 5000 mg/kg no mortality was observed. Hypopnea and lethargy were observed approximately 5 hours after dosing in the 2000 and 5000 mg/kg groups. Based on the mortality pattern, an LD_{50} could not be calculated using the method of Weil.

b. Preliminary Test Phase II

Thidiazuron technical was administered i.p. to Specific Pathogen Free COBS CD-1 (1CR) BR (1CR derived) mice. Test material was administered in 1% carboxymethylcellulose at concentrations of 0, 256, 320, 400 and 500 mg/ml (corresponding to 0, 2650, 3200, 4000 and 5000 mg/kg) to 5 animals/sex/dose level. Animals were observed for 168 hours after dosing.

Results:

Three mortalities were observed at 2650, 1 at 3200, 4 at 4000 and 1 at 5000 mg/kg. Based on the mortality pattern, an LD_{50} could not be calculated using the method of Weil.

6

c. Main Test

Thidiazuron technical was administered i.p. to Specific Pathogen Free COBS CD-1 (ICR) BR (ICR derived) mice. Test material was administered in 1% carboxymethylcellulose solution at a concentration of 200.0 mg/ml (corresponding to 2000 mg/kg) twice, with an interval of 24 hours between dosings. The vehicle control group was exposed to 1% carboxymethylcellulose solution (10 ml) and the positive control group received 4 mg/kg (twice) of Mitomycin C. Fifteen males and 15 females were used in each dose group.

Five males and 5 females were killed by cervical dislocation at intervals of 24, 48 and 72 hours after initial dosing from each group. A bone marrow smear was made from the femur of each animal onto a slide containing a drop of calf serum. The slides were air-dried, fixed in methanol and stained with Giemsa for 10 minutes. Slides were examined microscopically to determine the number of micronucleated cells per 1000 polychromatic cells and the ratio of normochromatic to polychromatic erythrocytes.

Results:

No clinical reactions or mortalities were observed after test compound administration. Mean numbers of micronucleated polychromatic cells were less than concurrent controls for both males and females at each interval. Positive control values were significantly greater ($p < .001$) than vehicle control values. The ratio of normochromatic to polychromatic erythrocytes was somewhat elevated at each interval, compared to the vehicle control, although the elevation attained statistical significance only at the 48 hour interval ($p < .001$).

Thus, no increase in micronucleated cells was observed in animals administered 2000 mg/kg thidiazuron. However, bone marrow depression was suggested by the increased ratio of normochromatic to polychromatic erythrocytes. To further investigate this finding, a follow-up study was conducted.

d. Follow-up Study

Thidiazuron technical was administered i.p. to Specific Pathogen Free COBS CD1 (1CR) BR (1CR derived) mice. Test material was administered in 1% carboxymethylcellulose solution at concentrations of 0, 2.4, 24 and 120 mg/ml (corresponding to 0, 24, 240 and 1200 mg/kg). Fifteen animals/sex/dose were used; 1% carboxymethylcellulose was used as a vehicle control and Mitomycin C was used as a positive control (2 mg/kg).

Thirty, 48 and 72 hours after the single dosings, five animals/sex/dose level were killed by cervical dislocation. A bone marrow smear was made from the bone marrow of the femur of each animal onto a slide containing a drop of calf serum. The slides were air-dried, fixed in methanol and stained with Giemsa for 10 minutes. Slides were examined microscopically to determine the number of micronucleated cells per 1000 polychromatic cells and the ratio of normochromatic to polychromatic erythrocytes.

Results:

No clinical reaction or mortalities were observed after test compound administration. The incidence of micronucleated cells in Thidiazuron treated groups was similar to the vehicle control group.

The incidence of micronucleated cells for Mitomycin C treated animals was significantly ($p < .001$) increased at 30 and 48 hours after dosings compared to controls. The ratios of normochromatic to polychromatic erythrocytes were similar in Thidiazuron treated groups and the vehicle control group. The ratio was significantly elevated in Mitomycin C treated animals at each interval ($p < .001$).

Classification: Acceptable

Protocol was similar to that recommended by Schmid* and no major deficiencies in study execution were apparent. The test compound does not appear to induce the formation of micronuclei. Bone marrow depression was suggested at a dose of 2000 mg/kg (2x) but was not observed at single doses of 1200 mg/kg or less.

*Schmid, W., "The Micronucleus Test", in Handbook of Mutagenicity Test Procedures, Kilbey, Legator, Nichols and Ramel (eds.), Elsevier Co., Amsterdam, 1979, pp. 235-243.

3. Teratology, Rat(1). Performed by Reprotox GmbH, Munster, West Germany (Project Number 413/A) and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois. Submitted on May 29, 1981.

Specific Pathogen Free rats of the Wistar Han 78 strain were mated (4 females to 1 male) overnight. Females with evidence of having mated were then transferred to individual cages and the date of positive indication of mating was considered day 0 of pregnancy. Twenty-nine females were assigned to the control group, 30 to the 100 mg/kg group, 30 to the 300 mg/kg and 30 to the 900 mg/kg groups. Technical thidiazuron was suspended in 1.0, 3.0 or 9.0% carboxymethylcellulose solution and administered by gavage on days 6-15 of pregnancy. Dams were observed daily and weighed on days 0, 6, 15 and 19 of pregnancy.

On day 19, dams were killed (by chloroform inhalation) and examined grossly. The ovaries and uteri were excised and the number of corpora lutea, live young, resorptions, fetal weights and the incidence of external fetal abnormalities were recorded. Uteri were examined for evidence of implantation. Approximately two thirds of litters were examined for skeletal abnormalities and one third were examined for visceral abnormalities using the method of Wilson.

Results:

Five animals died during the course of the study - one animal at 300 mg/kg and four at 900 mg/kg. Three of these deaths were likely to be due to intubation error and two (at the high dose) were probably compound-related. Body weight gain was depressed only in the 900 mg/kg group.

An unusually high incidence of total loss of litter was observed in treated animals (16, 7 and 8 in the 100, 300 and 900 mg/kg groups compared to 1 incident in the control group). It is noted that a dose relationship was not apparent in the number of dams with total litter loss and that litter losses appeared to be due primarily to death of the postimplantation fetus (percentage of post implantation losses were 82.9, 39.5 and 53.5 for 100, 300 and 900 mg/kg groups, respectively compared to 9.5 in the controls). Litter sizes were significantly reduced for all treated groups ($p < .05$) and mean fetal weight was significantly reduced for the high dose group only.

Although no increases were observed in external, visceral or skeletal abnormalities in treated animals, the small number of viable fetuses in treated groups precludes drawing a conclusion relating to teratogenic potential.

Core Classification: Supplementary Data

Because the occurrence of fetolethality was observed to a much greater extent in all treated groups compared to controls, a NOEL was not established in this study. Maternal toxicity was evident only at 900 mg/kg. The study results are inconclusive regarding fetotoxic and teratogenic potential.

4. Teratology, Rat(2). Performed by Reprotox GmbH, Munster, West Germany (Project Number 413/A) and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois. Submitted on May 29, 1981.

Specific Pathogen Free rats of the Wistar Han 78 strain were mated (4 females to 1 male) overnight. Females with evidence of having mated were then transferred to individual cages and the date of a positive indication of mating was considered day 0 of pregnancy. Twenty-five females were assigned to groups which received either 0, 25, 50, 100 or 300 mg/kg of thidiazuron technical, suspended in Myrj 53 solution, on days 6-15 of pregnancy.

On day 19, dams were killed (by chloroform inhalation) and examined grossly. The ovaries and uteri were excised and the number of corpora lutea, live young, resorptions, fetal weights and the incidence of external fetal abnormalities were recorded. Approximately two thirds of the litters were examined for skeletal abnormalities and one third were examined for visceral abnormalities using the method of Wilson. Uteri were examined for evidence of implantation using the technique of Salewski. Dams were observed daily and weighed on days 0, 6, 15 and 19 of pregnancy.

Results:

No parental animals died during the course of the study. No clinical observations of toxicity were noted. A slightly decreased mean body weight gain was observed in the females of the 300 mg/kg group.

Litter size, rate of implantation, pre and post-implantation losses were not affected by treatment. Mean fetal weight was significantly decreased only in the 300 mg/kg group. No visceral abnormalities were observed in any group. A single fetus with a "malformed head" was observed and the incidence of fetuses with "minor skeletal anomalies" (variations) were slightly increased in the high dose group (not significant, $p > 0.05$). Variations consisted primarily of delayed ossification of the occipital or parietals.

10

Core-Classification: Core Minimum.

Although maternal toxicity was only marginal at 300 mg/kg, the previous study indicated that frank maternal toxicity is to be expected at 900 mg/kg. The two studies, taken together, cover an adequate range of dose levels for the assessment of teratogenic potential. Fetotoxicity (decreased fetal weight) was observed only at 300 mg/kg and evidence of teratogenicity was not observed.

5. Teratology, Rabbit (1). Performed by Hazleton Laboratories Europe Ltd, Harrogate, England (Report No. 2282-14/2) and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois. Submitted May 29, 1981.

New Zealand White rabbits were mated (each doe with a maximum of 3 bucks) to achieve coitus, after which does were injected with chorionic gonadotropin to ensure ovulation. Sixteen females were then assigned to groups to receive the following dose levels of Thidiazuron technical on day 6-18 of gestation; 0, 25, 75 and 250 mg/kg/day by gavage. Test material was dissolved in 1% methylcellulose and 10 ml/kg were administered per animal.

All animals were observed daily after mating. Body weights were recorded on days 0, 3, 6, 9, 12, 15, 18, 21, 24 and 28 of gestation. Food consumption was recorded on day 3, 6, 9, 12, 15, 18, 21, 24 and 28 of gestation. On days 28, all surviving animals were killed by cervical dislocation and necropsied. The ovaries and uteri were excised and the number of corpora lutea, fetuses (live and dead), implantations, individual fetal weights, crown-rump lengths and sex of the fetuses were recorded.

Fetuses were examined for external defects and examined viscerally. Carcasses were fixed in "70% industrial methylated spirits" for 24 hours and the heads were then sliced through the frontoparietal suture line and examined for visible abnormalities. Following the examination of the head, the carcasses were placed in a 1% aqueous potassium hydroxide/.005% Alizarin Red S solution, processed with glycerol and examined for skeletal defects.

Results:

(Clarification of the procedure used for the examination of skeletal structure, especially the head, was requested by the reviewer and was received on April 23, 1982. See Attachments following this review).

The pattern of parental mortality did not appear to be influenced by the administration of test compound (6, 1, 6 and 0 deaths among the control, 25, 75 and 250 mg/kg groups). No reported clinical observations could be associated with the administration of test compound. All treated groups showed somewhat less weight gain than controls (15.0, 13.2, 12.6 and 10.0% body weight gain in the controls, 25, 75 and 250 mg/kg/day) although only the high dose animals were significantly affected.

Mean fetal weights and length were similar in all groups. The percentage of animals mated that subsequently became pregnant was slightly less for the high dose group (90.9, 93.3, 90.9 and 75% for the 0, 25, 75 and 250 mg/kg groups, respectively). Implantation rates per litter were similar in each group. Intrauterine deaths (post-implantation losses) were elevated in each treated group (10, 17.8, 16.5 and 30.5% of implantations in the 0, 25, 75 and 250 mg/kg).

Core Classification: Supplementary Data.

A NOEL for fetotoxicity was not established.

6. Teratology, Rabbit (2). Performed by Hazleton Laboratories Europe, Ltd., Harrogate, England (Report No. 2458-14/4) and submitted by Nor-Am Agricultural Products, Inc., Naperville, Illinois. Submitted on May 29, 1981.

New Zealand White rabbits were paired (each doe with a maximum of 3 bucks) to achieve coitus, after which does were injected with chorionic gonadotropin to ensure ovulation. Sixteen females were then assigned to groups to receive the following dose levels of Thidiazuron technical on days 6-18 of gestation; 0, 2.5, 7.5, 25, 75 and 250 mg/kg/day by gavage. Test material was dissolved in 1% Myrj 53 solution 0.9% saline and 10 ml/kg were administered per animal.

All animals were observed daily after mating. Body weights and food consumption were recorded on days 0, 3, 6, 9, 12, 15, 18, 21, 24 and 28 of gestation. On day 28, all surviving animals were killed by cervical dislocation and necropsied. The ovaries and uteri were excised and the number of corpora lutea, fetuses (live and dead), implantations, individual fetal weights, crown-rump lengths and sex of the fetuses were recorded.

Fetuses were examined for external defects and all fetuses were examined viscerally. Carcasses were fixed in "70% industrial methylated spirits" for 24 hours and the heads were then sliced through the frontoparietal suture line and examined for visible abnormalities. Following the examination of the head, the carcasses were placed in a 1% aqueous potassium hydroxide/.005% Alizarin Red S solution, processed with glycerol and examined for skeletal defects.

Results:

The pattern of parental mortality did not appear to be influenced by the administration of test compound (3, 3, 2, 5, 2 and 4 deaths in the 0, 2.5, 7.5, 25, 75 and 250 mg/kg groups, respectively. No reported clinical observations could be associated with the administration of test compound. Although body weight gain was slightly

12

decreased for all groups administered test compound, only the high dose group was decreased to an extent which was clearly affected by test compound (15, 13.8, 14.7, 14.7, 14.8 and 12.3% weight gain in the 0, 2.5, 7.5, 25, 75 and 250 mg/kg groups, respectively). The % of animals mated that subsequently became pregnant was similar in all groups. The implantation rates and percentage of intrauterine deaths (post-implantation losses) were similar in all groups less than 250 mg/kg with an increase observed at the high dose level (intrauterine deaths were 19.1, 9.2, 19.8, 10.5, 10.1 and 33.6% of implantations for the 0, 2.5, 7.5, 25, 75 and 250 mg/kg groups, respectively).

Core Classification: Core-Minimum.

The NOEL for fetal and maternal toxicity is 25 mg/kg.

7. Chronic Oral, Rat. Conducted at IBT (IBT No. 8560-09631) and submitted by Nor-Am Agicultural Products, Inc. on December 12, 1980.

[On September 10, 1980 a validation of this study was conducted by Experimental Pathology Laboratories and a classification of "Invalid" was assigned. Based on additional information submitted by the registrant on May 5, 1981, EPL prepared an addendum to the original validation. The addendum upgraded the classification to "Supplementary Data" and noted that "... there is additional toxicologic information such as effects on body weight, survival, clinical signs, hematology and clinical chemistry which do provide useful information about the the chronic toxicity of SN 49537."

Deficiencies that were noted in the addendum related primarily to histopathology. The validation notes that "... there was an excessive number of tissues not examined microscopically" and that "Failure to report the severity of microscopic alterations made it impossible to determine if administration of the compound was responsible for exacerbation of naturally occurring lesions which frequently is a manifestation of toxicity." Other deficiencies were noted in the presentation of neoplastic pathology data and the excessive mortality which necessitated the early termination of the study (greater than 50% at 18 months for all groups of males).]

Thidiazuron technical (Batch No. 251201 B00000 and 261103 B00000) was fed to Charles River Albino rats at dose levels of 0, 40, 120 and 500 ppm in the diet. Fifty animals per sex per dose level were used. Animals were observed monthly after week 20 until month 8, twice monthly until test day 514, and then daily until day 730. Food consumption was measured individually for 10 animals, or all surviving if less than 10, for each sex and group for the first 13 weeks, and one week per month for months 4 through 24. Animals were weighed weekly for the first 13 weeks and monthly for the remainder of the study. Blood samples were collected by suborbital sinus puncture and were collected from 10 rats/sex of the control and T-III group at 3, 6, 12 and 18 months, all surviving males of the T-III group (unfasted) after 21 months of testing, and 10 T-I, 10 T-II males (unfasted), 10 control and 10 T-III females prior to sacrifice after 102 weeks. Samples were analyzed for leukocyte count, RBC count, Hb, hematocrit, MCV, MCH, MCHC, platelet count, differential leukocyte count, blood glucose, BUN, SAP, SGPT, SGOT, cholesterol, protein and albumin/globulin ratio.

Urine samples were collected from the same animals at the same intervals with the exception of males after 18 months, for whom data was not collected. Urine was analyzed for glucose, albumin, bilirubin, ketones, pH, specific gravity and microscopic elements present.

Gross necropsies were conducted on all animals, excluding those severely autolyzed. Animals were sacrificed at the following times:

a. T-III males on day 637
b. Control, T-I and T-III on days 705 and 706
c. All females on day 731 and 732

Animals were sacrificed by CO_2 asphyxiation and immediately exsanguinated. Weights of adrenals, brain, gonads, heart, kidney, liver, spleen and thyroid were recorded. "Sections of all available tissues and organs of all rats in the control and high dose groups were made into slides and examined histologically. Selected organs and tissues from rats in the low (40 ppm) and middle (120 ppm) dose groups were made into slides and examined histologically. The organs and tissues selected were those which showed tissue masses or lesions suggestive of possible neoplasms by gross examination."

Results:

(Diet analysis data indicates that animals received the following average amounts of test compound in the diet; control 1.7 ppm, T-I 40.3 ppm, T-II 120.9 ppm, T-III 477.7 ppm.)

Body weights of T-III females were consistently lower than control females, male body weights did not appear to be affected by test compound exposure. Mortality of both T-III males and females was somewhat greater than control animals over the course of the study (through 21 months, 39 (78%) control males had died vs. 45 (90%) T-III males, through 24 months, 37 (74%) control females had died vs. 44 (88%) T-III females.

No effects considered to be compound related were observed on food consumption, hematology, clinical chemistry, urinalysis, or organ weights.

Available observation data and histopathology data did not suggest compound related effects but further confirmed the presence of respiratory disease.

Core-Classification: Supplementary Data.

Available data suggested a compound related effect on mortality (in both males and females) and body weight (females only) in T-III animals. Although no effects were observed at dose levels less than 500 ppm, this study does not permit the establishment of a NOEL due to the deficiencies noted in the validation regarding histopathology.

8. Oncogenicity, Mouse. Conducted at IBT (IBT No. 8580-10725) and submitted by Nor-Am Agricultural Products, Inc. on December 12, 1980.

("This study was validated for the Agency by Experimental Pathology Laboratories on April 29, 1982 and classified as "Valid". The following three minor deficiencies were noted during the validation.

1. High dose females were terminated at 18 months on test. All other females were sacrificed after 22 months on test.

2. Mammary gland and parathyroid were not routinely collected for histological examination, although their collection was specified in the protocol.

3. Several deficiencies related to the thoroughness of clinical observations.")

Swiss White mice of unknown age (body weights ranging from 22.5 to 27.6 grams) were assigned to test groups as follows:

	Males	Female	Level (ppm)
Vehicle Control	50	50	0
T-I	50	50	50
T-II	50	50	250
T-III	50	50	1000

Treated groups received thidiazuron technical, Batch Number 261103B0000, 100% purity for periods ranging from 18 months (T-III females) to 22 months (control, T-I and T-II females). Test material was mixed with acetone and added to stock feed to achieve the desired dietary concentration. Body weihgts were recorded on a monthly basis. Food consumption was measured one week of each month. Observations were recorded 5 days a week (not including weekends or holidays). The following hematologic studies were conducted on 11 female mice from the T-III group on test day 560: total leukocyte count, erythrocyte count, hemoglobin, MCV, MCH, MCHC and differential leukocyte count.

A gross pathologic examination was conducted on all animals on test. Microscopic examination was conducted on tissues of all animals (when not precluded by autolysis) and in addition, to those tissues appearing grossly abnormal, the following tissues were examined:

Heart
Liver
Lung
Pancreas
Stomach (cardia, fundus, pylorus)
Small intestine (duodenum, jejunum and ileum)
Caecum
Colon
Spleen
Kidneys
Urinary bladder
Testes
Ovaries
Prostate gland
Uterus
Epididymides
Gall bladder
Seminal vesicles
Pituitary gland
Adrenal glands
Salivary glands (sublingual, parotid, submandilunar)
Lymph nodes (cervical and mesenteric)
Thyroid gland
Parathyroid glands
Skeletal muscle
Sternum with marrow
Peripheral nerve (sciatic)
Trachea
Spinal cord
Eyes with optic nerve
Brain (cerebrum, cerebellum and pons)
Aorta
Esophagus

Results:

No histological lesions or tumors were observed which could be associated with compound exposure. The most common histological findings were either artifactual i.e., congested livers resulting from not being bled prior to examination or were common to aging mice i.e., amyloidosis. The most frequently occurring tumor type was adenoma of the lung (4, 8, 2, and 5 animals in the control, 50, 250 and 1000 ppm groups, respectively); the incidences of all other tumor types were low (three or less per group) and similar in control and treated groups. Body weight gains were similar in all test groups, although slightly less in high dose males and females compared to controls. Mortality was slightly higher among treated males (29, 36, 40 and 39 deaths through final sacrifice for the control, 50, 250 and 1000 ppm groups) but the increase was not dose related and could not be associated with compound exposure. Skin lesions were somewhat increased in treated females, (6, 12, 15 and 15 females with lesions in the control, 50, 250 and 1000 ppm group) but histological examination of the lesions found them to be changes considered to be spontaneous and common in mice (such as ulcerative dermatitis, acanthosis, parakeratosis and hyperkeratosis). Hematology conducted on the T-III females on day 560 revealed no remarkable findings (based on a comparison with historical control values for Charles River CD-1 females, personal conversation with Dr. Lee of Charles River Breeding Laboratories, Wilmington, MA, April 27, 1982).

Conclusion:

Although an MTD was not demonstrated it is likely that the high dose (1000 ppm) approaches the MTD based on slightly decreased body weight gains in both males and females. In addition, administration of thidiazuron at levels of 600 ppm and greater for periods of more than 90 days has shown an effect (decreased weight gain) in rats and dogs. The differences in termination dates of the female test groups (22, 22, 22 and 18 months for the control, 250, and 1000 ppm groups, respectively) makes comparison of tumor incidence in control and high dose females difficult. However, based on the very low incidence of tumors observed in high dose females after 18 months and the comparable incidences of tumors in control, low and mid dose females after 22 months at each tissue site, this reviewer considers it unlikely that the shorter duration of the T-III female exposure period has concealed an oncogenic effect. It is also noted that a thorough validation of the gross and histopathologic examination has determined that gross lesions and clinical observations of possible tumor masses were adequately followed up histologically.

Core Classification: Core-Minimum

17

9. Ninety Day Oral, Rat. Conducted at IBT (IBT No. 8560-08337) and submitted by Nor-Am Agricultural Products on May 27, 1976.

(This study was validated by Dr. Henry Spencer on July 3, 1979 and classified as "Valid".)

Charles River albino rats were exposed to technical thidiazuron at dose levels of 0, 60, 200 and 600 ppm in the diet for a period of 90 days (15 males and 15 females per dose level). Food consumption was measured for 10 animals of each sex at each dose level on a weekly basis. All animals were weighed on a weekly basis. Blood and urine samples were collected after 45 and 84 days on test and were analyzed for (blood) total leukocyte count, erythrocyte count, Hb, Ht, differential leukocyte count, MCV, MCH, MCHC, blood glucose, BUN, SAP, SGPT and (urine) glucose, albumin, pH, specific gravity and microscopic elements. After 90 days, all surviving rats were rendered unconscious by CO_2 exposure and exsanguinated. All animals were necropsied and the weights of brain, gonads, heart, kidneys, liver and spleen were recorded. Tissues of the heart, aorta, lungs, trachea, liver, spleen, lymph nodes, pancreas, esophagus, stomach, small and large intestines, kidneys, urinary bladder, pituitary, thyroid, parathyroid, adrenal, gonads, brain, spinal cord, peripheral nerves, eyes, optic nerve, salivary glands, sternum, bone, skeletal muscle and bone marrow were examined from 10 animals per sex of the control and T-III groups.

Results:

No mortalities were observed in any group during the course of the study. Neither body weight nor food consumption were significantly affected by treatment. Hematology, urinalysis and clinical chemistry parameters were also not affected by treatment. The following organ weights of high dose animals were significantly different than controls: gonads of females (significantly heavier, $p < .01$), male heart (significantly lighter, $p < .05$), and male spleen (significantly lighter, $p < .05$). No gross or histological lesions were observed which could be associated with the observed organ weight differences, or, more generally, with exposure to thidiazuron.

Core Classification: Core-Minimum

Although more extensive histological examination and a larger number of animals at each dose level i.e. at least 20 animals/sex would have been preferable, this study meets minimum standards of acceptability. Based on organ weight differences noted at the high dose level (600 ppm), the NOEL in this study is 200 ppm.

10. Ninety Day Oral, Dog. Conducted at Industrial Biotest Labs (IBT No. 8531-08338) and submitted by Nor-Am Agricultural Products on May 27, 1976.

(This study was validated by Dr. Henry Spencer of Toxicology Branch on July 3, 1979. No discrepancies were noted.)

Beagle dogs, approximately 5 1/2 months of age, were exposed to technical thidiazuron at dose levels of 0, 100, 300 and 1000 ppm in the diet. Four males and 4 females were used at each dose level. Animals were fed ad libitum for 5 hours each day and food consumption was measured on a weekly basis. Animals were examined daily for clinical signs and symptoms of toxicity. Blood and urine samples were collected after 42 and 85 days on test and were analyzed for the following (blood): leukocyte count, erythrocyte count, Hb, Ht, MCV, MCH, MCHC, differential leukocyte count, BUN, glucose, SAP, SGOT, SGPT, (urine) albumin, glucose, pH, specific gravity and microscopic elements. After 90 days, animals were anesthelized by i.v. injection of ketamine hydrochloride and pentobarbital and killed by exsanguination.

All animals were necropsied and the weights of the liver, kidneys, heart, brain, spleen, gonads, adrenals, thyroids and pituitaries were recorded. The following tissues were examined from all animals:

Aorta
Heart
Trachea
Lung
Lymph nodes (cervical, mediastinal, mesenteric)
Spleen
Liver
Thyroid gland
Parathyroid glands
Kidney
Urinary bladder
Testes (Males)
Epididymides (Males)
Prostate gland (Males)
Ovaries (Females)
Uterus (Females)
Bone marrow (sternum)
Muscle (skeletal)
Brain

19

Adrenal glands
Pituitary gland
Pancreas
Salivary gland (submaxillary)
Esophagus
Stomach
Small intestine (duodenum, jejunum, ileum)
Spinal cord
Peripheral nerve (sciatic)
Eyes
Optic nerve

Results:

No mortalities were observed at any dose level. Body weight gain of high dose males was less than that of controls (1.5 kg for T-III males vs. 3.1 kg for controls). Female body weights were not affected by treatment and food consumption was similar for all groups in both sexes. Hematology, clinical chemistry and urinalysis were not affected by treatment.

No organ weight differences were observed which could be associated with treatment. Gross and histological examination revealed no compound-related alterations. Seventeen of the 36 dogs were diagnosed as having roundworm infections.

Core Classification: Core-Minimum

Based on decreased body weight gain in high dose males, the NOEL is 300 ppm in this study.

Gary J. Burin FDC 4/30/82

Gary J. Burin, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

TS-769:th:TOX/HED:GBurin:4-28-82:card 3

20

CFR 180. 0 Dropp 10/24/78

File last updated 10/24/76

ACCEPTABLE DAILY INTAKE DATA

Dog NOEL		S.F.	ADI	HPI
mg/kg	ppm		mg/kg/day	mg/day/60kg
7.500	300.00	2000	0.0038	0.2250

Current Action PP6G1807

CROP	Tolerance	Food Factor	mg/day/1.5kg
Cottonseed(41)	0.200	0.15	0.00045
Milk&Dairy Products(93)	0.050	28.62	0.02146
Eggs(54)	0.100	2.77	0.00416
Meat,inc poultry(89)	0.200	13.85	0.04154

HPI	TMRC	% ADI
0.2250 mg/day/60kg	0.0676 mg/day/1.5kg	30.05

****

21

ATTACHMENT

NOR-AM

NOR-AM AGRICULTURAL PRODUCTS, INC. 350 WEST SHUMAN BOULEVARD NAPERVILLE, ILLINOIS 60566 PHONE (312) 961-6500 TWX 910-651-0201

April 23, 1982

Dr. Larry Chitlik
HED, EPA
Crystal Mall #2
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Dr. Chitlik:

Enclosed please find 3 copies of telex from Hazleton Laboratories Europe LTD in answer to my telex (copies enclosed) asking for details concerning the examination of the skulls in rabbit teratology studies with thidiazuron (Hazleton studies 2282 - 14/2 and 2458 - 14/4).

Yours sincerely,
NOR-AM AGRICULTURAL PRODUCTS, INC.

Margareta Lambert
Supervisor, Technical Services

ML:m

ATTACHMENT

NOR-AM
NOR-AM NPVL

RCA APR 13 1007
TUUET HAZLAB G

6603 13-APRIL 1982

NOR - AM

ATT: MARGARETA LAMBERT

RE: YOUR TELEX APRIL 8, 1982

RABBIT FOETAL SKELETAL EXAMINATION INCLUDES INSPECTION OF THE SKULL. ALL FOETUSES ARE SKINNED, DISSECTED AND THE VISCERA EXAMINED. THEY ARE EVISCERATED AND FIXED FOR 24 HOURS IN I.M.S. THE SKULL OF EACH FOETUS IS CAREFULLY SLICED ONCE THROUGH THE LINE OF THE FRONTOPARIETAL SUTURE AND THE BRAIN EXAMINED FOR VISIBLE ANOMALIES. THE CARCASS, INCLUDING THE SKULL IS THEN PROCESSED (STAPLES) AND ALL BONES EXAMINED FOR SKELETAL DEFECT USING APPROPRIATE VIEWING AIDS.

ALL THE SKULL BONES (NASAL MAXILLA, FRONTAL, PARIETAL, ZYGOMA, OCCIPITAL, SQUAMOSAL, INTERPARIETAL, TYPANIC ANNULUS AND SPHENOID) ARE EXAMINED FOR SIZE, FORMATION, POSITION AND VARIATIONS IN DEGREE OF OSSIFICATION.

REGARDS
PETER SIMONS

HAZLETON LABORTORIES EUROPE LTD, HARROGATE
57735 HAZLAB G
NOR-AM NPVL

REPLY TO THIS TELEX VIA RCA

23