CASWELL FILE

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

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SEP 28 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of a 1 year dog study, an in vitro cytogenetic assay in human lymphocytes and a 104 week chronic toxicity/oncogenicity in rats with Thidiazuron for conditional registration requirements.
EPA ID # 45639-89; EPA Record # 174285; EPA Accession #'s 262815, 262816, 262817, 262818 and 262819; Caswell #659A; Tox Branch Project 1791.

TO: Richard F. Mountford (PM #23)
Herbicide - Fungicide Branch
Registration Division (TS-767C)

FROM: Stephen C. Dapson, Ph.D. Stephen C. Dapson 9/22/87
Pharmacologist, Review Section V
Toxicology Branch/HED (TS-769C)

THRU: Quang Q. Bui, Ph.D., D.A.B.T. Quang Q Bui 9/23/87
Acting Section Head, Review Section V
and
Theodore M. Farber, Ph.D., D.A.B.T. 9/27/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Registrant: NOR-AM Chemical Company
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Wilmington, Delaware 19803

Action Requested: Review Thidiazuron conditional registration requirements: 1-year dog; in vitro cytogenetic assay: human lymphocytes; 104-week chronic toxicity/oncogenicity: rat.

Recommendations: For the chronic toxicity study in dogs (Schering Study No. TX 83.003): Under the conditions of the study, SN 49 537 (Thidiazuron) fed to beagle dogs for 1 year at levels of 100, 300 or 1000 ppm produced compound-related hemolytic anemia in mid and high dose animals. There were increased absolute and relative liver, spleen, and lymph node weights in males receiving 300 and 1000 ppm. Increases in spleen weights of mid dose females were noted, as well as increases in liver and lymph node weights of the high dose females. Histologically, there was increased incidence of pigment deposition in liver, kidney and spleen of mid and high dose males and females as well as an increase in Kupffer cells.

The Lowest Observed Effect Level (LOEL) for systemic toxicity is 300 ppm SN 49 537 (Thidiazuron) in the diet and the No Observed Effect Level (NOEL) for systemic toxicity with Thidiazuron in the diet is 100 ppm.

This study is classified as Core-Supplementary Data since histological examination for all tissues was not performed on low and mid dose animals. The classification may be upgraded upon submission and acceptance by the Agency of these data. Further, the hemolytic anemia and effects on certain organs may be indicative of immunotoxicity from this compound and may require further study for clarification of this effect.

For the mutagenicity study: in vitro cytogenetic study with human lymphocytes (Litton Bionetics No. LBI 20990): It is recommended that the nonactivated assay be repeated with an appropriate level of the positive control and with a human lymphocyte system that has demonstrated ability to detect clastogenic responses. It is also recommended that either separate experiments with lymphocytes from different donors or replicate cultures from different donors be included in the study.

Thidiazuron was tested under nonactivated and S9-activated conditions. The nonactivated assay was conducted with 2.5, 5.0, 10.0, 20.0 and 40.0 ug/ml of Thidiazuron and produced equivocal negative results because of the borderline activity of the positive control (mitomycin C at 0.25 ug/ml). Also, the presence of rare complex chromosome aberrations was found at 5 and 20 ug/ml. The nonactivated assay is Unacceptable because the sensitivity of the system for detecting a direct-acting clastogen is questionable.

In the presence of S9 activation, five doses (25 to 300 ug/ml) of Thidiazuron did not induce a clastogenic response. Thidiazuron was cytotoxic at 300 ug/ml. The positive control (cyclophosphamide at 120 ug/ml) in the S9-activated assay induced a statistically significant response (indicating the activated assay had an adequate level of sensitivity). Thidiazuron in the S9-activated assay was assayed up to a cytotoxic level with no effect. The S9-activated assay is Acceptable.

For the chronic toxicity and oncogencity study in rats (RCC Project 011924): Thidiazuron was fed to Wistar (KFM-Han) rats at dietary levels of 0, 70, 200 or 600 ppm. There were no overt signs of toxicity or dose related effects on mortality, pharmacotoxic signs, clinical laboratory findings, absolute or relative organ weights and oncogenic changes at any site. There were slight, sometimes statistically significant decreases in body weight and food consumption in the 600 ppm males when compared to the control group. No changes in body weight or food consumption were noted for females at any dose level.

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The LOEL for systemic toxicity in male rats is 600 ppm (35.9 mg/kg/day) of Thidiazuron in the diet based on minimal changes in body weight and food consumption. The NOEL for systemic toxicity in male rats is 200 ppm (11.8 mg/kg/day) of Thidiazuron in the diet and 600 ppm of Thidiazuron in the diet for female rats.

This study is classified as Core-Minimum Data for chronic toxicity, however, the Maximum Tolerated Dose (MTD) was not achieved, therefore, this study is classified as Core-Supplementary Data for oncogenicity. Further testing is required for assessment of possible oncogenic effects.

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EPA: 68-02-4225
DYNAMAC No. 255-A
July 9, 1987

DATA EVALUATION RECORD

THIDIAZURON (SN 49 537)

Chronic Toxicity Study in Dogs

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-9-87

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DATA EVALUATION RECORD

THIDIAZURON (SN 49 537)

Chronic Toxicity Study in Dogs

REVIEWED BY:

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Acting EPA Section Head

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Date: 9-22-87

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DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic toxicity study in dogs.

ACCESSION NUMBER: 262815.

TEST MATERIAL: Thidiazuron (SN 49 537).

SYNONYMS: 5-N-phenylcarbamoylamino-1,2,3-thidiazol.

STUDY NUMBER(S): TX 83.003.

SPONSOR: Schering AG, Department for Experimental Toxicology, Berlin, West Germany.

TESTING FACILITY: Main Department of Experimental Toxicology, Schering AG, Postfach 650311, Müllerstrasse 170-178, D-1000, Berlin 65, West Germany.

TITLE OF REPORT: SN 49 537: Systemic Tolerance Study in Dogs Following Daily Administration Via the Feed Over a Period of One Year.

AUTHOR(S): Schuppler, J., and Khater, A. R.

REPORT ISSUED: October 17, 1985.

CONCLUSIONS:

Under the conditions of the study, SN 49 537 fed to beagle dogs for 1 year at levels of 100, 300, or 1000 ppm produced compound-related hemolytic anemia in mid- and high-dose animals. The severe anemia resulted in moribund sacrifice of a high-dose male and removal of a high-dose female from dosing. There were increases in the absolute and relative weights of liver, spleen, and lymph nodes in males receiving 300 and 1000 ppm. Increases in spleen weights in mid-dose females as well as liver and lymph node weight in high-dose females were found. Histologically, there was an increased incidence of pigment deposition in liver, kidney, and spleen of mid- and high-dose males and females as well as an increase in Kupffer cells. The LOAEL for systemic toxicity is 300 ppm SN 49 537 in the diet and the NOAEL is 100 ppm.

Core Classification: Core Supplementary since histopathologic examination for all tissues was not performed on low- and mid-dose dogs. The classification could be upgraded on submission of these data and on clarification of the impact of pneumonia on the outcome of the study.

A. MATERIALS: A copy of the materials and methods is included in Appendix A.

1. Test Compound: SN 49 537, a beige-colored powder from batch No. 7/9.82; purity: 98.7-98.9 percent SN 49 537; impurities [redacted]

2. Test Animals: Species: dog; strain: beagle; age: 4 to 6.5 months; weight: males--6.5 to 10.0 kg, females--5.9 to 9 kg; source: Winkelmann, address of breeder not provided in the report.

B. STUDY DESIGN:

1. Animal Assignment: After more than 2 weeks of acclimation, the animals were distributed into the following treatment groups:

Test group	Dose in diet (ppm)	Main study (12 months) Males	Females
1 Control	0	5	5
2 Low (LDT)	100	5	5
3 Mid (MDT)	300	5	5
4 High (HDT)	1000	5	5

Doses were based on a 3-month dog study (IBT Report No. 8531-08338 (Jan. 6, 1986), in which the only effect was a lowered body weight gain in two of four male dogs receiving a dietary level of 1000 ppm.

The animals were individually caged in an environmentally controlled room with a 12-hour light/12-hour dark cycle.

2. Diet Preparation: A single batch of SN 49 537 (7/9.82), with a determined average purity of 98.8 percent, was used to prepare the test diets throughout the study. Diets were prepared biweekly and stored at room temperature. Stability of test compound in feed was determined by analysis immediately after mixing the powdered diet with water to make mash and after 1 and 3 weeks of storage. Homogeneity of SN 49 537 in the test diets was determined at study initiation. The concentration of test compound in the diets was determined at bimonthly intervals throughout the study.

   Results: The mean analyzed dietary concentrations of the test compound were 90.1 (range, 77-121), 89.9 (80-108), and 89.2 (67-107) percent of nominal levels of 100, 300, and 1000 ppm, respectively. However, analytical determinations showed that the compound levels were 23 to 33 percent lower than the nominal concentrations at three intervals, i.e., once at the low dose and twice at the high dose; these deviations were not acceptable. The test compound in the diets was stable for 3 weeks, with analyzed recoveries ranging from 91 to 104 percent of the nominal values. The test values for homogeneity varied between 100 and 112 percent. The test compound was stable for about 1 year; two determinations (9 months apart) showed that its purity was 98.7 and 98.9 percent, respectively.

3. Animals received food (pulverized Altromin H diet) for 2 hours/day and water ad libitum.

4. Intergroup differences for the tested parameters were analyzed using Dunnett's test.

5. A quality assurance statement was dated October 16, 1985.

C. METHODS AND RESULTS:

1. Observations: Animals were observed twice daily on weekdays and once on weekends for moribundity, mortality, and signs of toxicity.

   Results: No spontaneous deaths occurred during the study. One high-dose male (No. 4744) exhibited signs of clinical toxicity (apathy, high heart frequency, anemia, inspiratory dyspnea) associated with low food consumption and reduced body weight. These signs were considered compound related. The animal was sacrificed moribund in study week 7. One high-dose female

(No. 4731) also showed similar signs of toxicity plus pale mucous membranes. After week 38, this dog was fed control diet for a reversibility study. The compound-related signs of pale mucous membranes disappeared 9 weeks after withdrawal of the test compound. This animal was sacrificed 4 months later than the scheduled terminal sacrifice. One mid-dose (male) and four high-dose animals (two of each sex) showed symptoms of anemia. These symptoms were more severe in male No. 4744 and female No. 4731. No compound-related clinical signs were observed in the low-dose animals.

Other nonspecific clinical conditions (conjunctivitis, corneal opacity, alopecia, eczema, diarrhea, emesis) occurred sporadically and were not considered compound related. These nonspecific signs were not recorded for individual animals.

2. Body Weight: Animals were weighed each week for 58 weeks.

Results: SN 49 537 administration caused no significant change in group mean body weights. Mean weight gains ranged between 2.1 and 2.8 kg over the 58 weeks of the study in all groups. Two high-dose animals had weight loss. High-dose male No. 4744 showed a weight loss of 2 kg between weeks 6 and 7 and was sacrificed moribund. High-dose female No. 4731 showed a gradual loss (1.2 kg) in body weight between weeks 35 and 38. After this animal was switched to the control diet for a reversibility study, body weight recovered within 4 weeks to a level similar to that of other high-dose females.

3. Food Consumption and Compound Intake: Individual food consumption was determined daily. The daily compound intake in mg/kg body weight was calculated.

Results: Except for two high-dose animals, SN 49 537 administration caused no significant change in the individual or group mean food consumption. A high-dose male (No. 4744) showed a 51 percent reduction in food consumption during week 6 and was sacrificed moribund in study week 7. Food consumption in a high-dose female (No. 4731) was decreased between weeks 35 and 38; the animal was removed from the main study during week 38 and given control diet thereafter.

In general, compound intake in the dosage groups remained fairly constant throughout the study. The calculated compound intake values (weeks 1 to 52) for the nominal dietary concentrations of 100, 300, and 1000 ppm in males were 3.93, 11.8, and 38.3 mg/kg/day and in females were 4.04, 11.1, and 36.0 mg/kg/day, respectively.

4. Ophthalmology: Ophthalmologic examinations were performed on all animals 2 weeks prior to study initiation and in weeks 12, 34, 40, and 51.

Results: Corneal opacity was found in one mid-dose male at weeks 12, 34, 40, and 51, in one low-dose female at weeks 34, 40, and 51, and in one mid-dose female at weeks 40 and 51. In other animals (control groups, two males and one female; high-dose groups, one male and one female), opacity was randomly found but did not persist. All corneal opacities were unilateral and were not considered compound related by the study authors.

5. Neurology: Neurological testing, corneal, gag, cough, flexor, and patellar reflexes; tactile reactions; and sensitivity of the skin and extremities, were performed on all animals 2 weeks prior to study initiation and during weeks 34 and 39.

Results: No compound-related effects in neurological functions were observed.

6. Hematology and Clinical Chemistry: Blood samples for hematology and clinical chemistry were collected from all animals 2 weeks before treatment and at weeks 1, 12, 27, 39, and 52. The CHECKED (X) parameters were examined in all the treatment groups.

a. Hematology

- X Hematocrit (HCT)†
- X Hemoglobin (HGB)†
- X Leukocyte count (WBC)†
- X Erythrocyte count (RBC)†
- X Platelet count†
- X Leukocyte differential count
- X Mean corpuscular HGB (MCH)
- X Mean corpuscular HGB concentration (MCHC)
- X Mean corpuscular volume (MCV)
- X Erythrocyte sedimentation rate
- X Reticulocyte count (RET)

In addition, hematologic determinations were performed on high-dose male No. 4744 4 days prior (week 6) to and on the day of moribund sacrifice (week 7) and on high-dose female No. 4731 during study weeks 10, 35, 36, and 38 and throughout the reversibility study.

Results: The authors reported statistically significant differences for red cell parameters in mid-dose males and in high-dose females when compared to controls. These were a decreased hematocrit and hemoglobin in mid-dose males at week 27, a decreased red cell count in the same group at weeks 27 and 39, and a decreased hematocrit (weeks 12 and 29) and hemoglobin (week 12) in high-dose females. In addition, there were nonsignificant decreases in these parameters in the same groups at other analysis intervals (Table 6, CBI p. 23). Review of the individual animal data indicated that extreme outlier values were included in calculating the means and in the statistical analysis.

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†Recommended by USEPA Subdivision F (October 1982) Guidelines.

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In addition, values for female No. 4731 were included at all intervals despite the fact that this animal did not receive test compound after week 36. When the outlier values were omitted there were not sufficient animals for meaningful statistical analysis of the data (e.g., only three high-dose females). All the remaining values for individual dogs, however, were within the normal range.

The severity of the anemia in affected animals (the two mid-dose males and two high-dose females) is illustrated in Table 1. The effect on red cell parameters was accompanied by an increase in reticulocytes and also an increase in leukocytes.

Mean platelet counts were increased in low- and mid-dose males at week 52 and decreased in high-dose females at weeks 12 and 27 (Table 2); however, these changes did not appear to parallel the severe anemic response in the individual dogs shown in Table 1.

b. Bone Marrow Studies: During the necropsy examinations, bone marrow (femoral) samples were collected from all the animals terminated at week 52 and high-dose male (No. 4744) sacrificed moribund at week 6. The CHECKED (X) parameters were examined. In addition, samples were used to prepare and evaluate myelograms.

X Blast cells	X Mast cells
X Erythropoiesis	X Reticulum cells
X Granulocytopoiesis	X Mitoses
X Monocytes, mature	X Undifferentiated cells
X Lymphocytes, total	X Nucleated bone marrow cells
X Plasma cells	
X Megakaryocytes	

Results: No intergroup differences in any of the determined parameters were observed. Both mid-dose male No. 4702 and high-dose female No. 4689 showed a severe anemic response (Table 1) and marked increase in compensatory erythropoiesis. The male also showed an increase in nucleated cells. High-dose male No. 4707, which did not show an anemic response, had an increase in hematopoiesis and increase in nucleated cells.

c. Coagulation Studies: In weeks 12 and 52, some factors (thromboplastin, activated partial thromboplastin and thrombin times, and fibrinogen) involved in blood coagulation were determined in all the treatment groups. These determinations were made on male No. 4744 at week 6 and on female No. 4731 in study weeks 10 and 38 and during week 14 of the reversibility study.

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†Recommended by USEPA Subdivision F (October 1982) Guidelines.

TABLE 1. Hematologic Values[a] in Affected Animals

Animal/Dose Parameter	Week:	-2	12	27	39	52
Male No. 4702/300 ppm						
HCT (%)		44	36	29	28	24
HGB (g/dL)		14.9	12.0	8.4	8.7	6.9
RBC (10^6/mm^3)		6.5	5.3	3.7	3.6	3.1
RET (No./1000 RBC)		28	16	78	276	114
Male No. 4728/300 ppm						
HCT (%)		44	36	28	22	36
HGB (g/dL)		14.9	12.0	8.4	7.0	12.0
RBC (10^6/mm^3)		6.5	5.3	3.7	2.8	5.3
RET (No./1000 RBC)		28	16	78	416	34
Female No. 4689/1000 ppm						
HCT (%)		46	12	36	31	37
HGB (g/dL)		14.9	4.2	10.9	9.9	11.7
RBC (10^6/mm^3)		6.2	1.7	4.6	3.7	4.9
RET (No./1000 RBC)		8	52	62	276	34

	Week:	-2	12	27	36	40	52	67
Female No. 4731/1000 ppm[b]								
HCT (%)		45	12	14	5	16	37	35
HGB (g/dL)		14.7	3.4	4.1	1.7	5.0	12.3	11.7
RBC (10^6/mm^3)		6.5	1.2	1.3	0.5	1.7	4.9	4.7
RET (No./1000 RBC)		2	232	176	180	150	18	0

	Week:	-2	5	6
Male No. 4744/1000 ppm				
HCT (%)		43	4	7
HGB (g/dL)		14.1	2	2
RBC (10^6/mm^3)		6.4	0.8	0.8
RET (No./1000 RBC)		141	19	284

[a]Abbreviations used are as follows:
HCT--hematocrit
HGB--hemoglobin
RBC--erythrocyte count
RET--reticulocyte count.

[b]This animal was taken off the test diet at week 36 for a recovery study.

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TABLE 2. Mean Platelet Values (x $10^3/mm^3$ ± SD) in Dogs Fed SN 49 537 for 52 Weeks

Study Week	Males (ppm)				Females (ppm)			
	0(5)[a]	100(5)	300(5)	1000(4)	0(5)	100(5)	300(5)	1000(5)
-2	353±74	402±52	401±59	401±29	311±32	328±46	309±31	307± 49
12	371±24	334±25	359±33	278±23**	356±47	307±47	334±33	260± 46**
27	325±41	291±37	288±53	288±56	340±21	343±27	337±49	224± 71**
39	400±36	416±40	441±35	325±75	439±62	412±54	439±43	357±103**
52	333±21	407±27**	426±41**	350±13	362±13	429±63	370±31	374± 62

[a] ( )—Number of animals. Values for 39 and 52 weeks include only four females. At week -2 there were five males.

**Significantly different from control value ($p < 0.01$), values were adapted from the CBI report.

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Results: A significant ($p < 0.01$) decrease of 14 percent in thromboplastin time in mid-dose females was not considered biologically important by the study authors. Compared to control values (ranging between 180 and 240 mg/dL), serum fibrinogen levels in the following three high-dose animals were very high:

Prior to sacrifice	Male No. 4744	453 mg/dL
At week 12	Female No. 4689	619 mg/dL
At week 12	Female No. 4731	377 mg/dL

Sedimentation rates (24 hour) for both above high-dose females were also increased from a pretest value of 12 to >150 mm at week 12 and also remained high at week 27. Male No. 4744 (sacrificed moribund) also had a 24-hour sedimentation rate >151 mm. The fibrinogen values in both females were returned to normal in week 52.

d. Clinical Chemistry: The CHECKED (X) parameters were examined.

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
	Phosphorus†	X	Cholesterol†
X	Potassium†	X	Globulins
X	Sodium†	X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)	X	Total protein†
	Cholinesterase		Triglycerides
	Creatinine phosphokinase†		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (SGPT)†		
X	Serum aspartate aminotransferase (SGOT)†		

Results: Significant ($p < 0.05$ or $p < 0.01$) compound-related changes in clinical chemistry parameters were mainly observed in the high-dose groups and to a lesser extent in the mid-dose groups (Table 3). Alkaline phosphatase levels were substantially increased when compared to controls in mid- (58 percent) and high-dose (72 percent) females at week 39 and in high-dose males (138 percent) and females (50 percent) at week 52. The albumin/globulin (A/G) ratio was decreased in mid- and high-dose males and females at week 52. At various determination intervals, levels of β- and γ-globulins (determined electrophoretically)

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†Recommended by USEPA Subdivision F (October 1982) Guidelines.

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TABLE 3. Summary of Selected Mean Clinical Chemistry Data (±SD) in Dogs Fed SN 49 537 for 52 Weeks

Parameter Determined	Study Week	Males (ppm)			Females (ppm)		
		0(5)[a]	300(5)	1000(4)	0(5)	300(5)	1000(5)[b]
Alkaline phosphatase (U/L)	39	93±25	77±18	143±35	81±16	128±30*	139±17**
	52	92±23	81±23	219±12**	82±11	105±24	123±20*
Total protein (g/dL)	27	5.8±0.1	5.6±0.2	5.9±0.3	5.5±0.1	5.6±0.2	6.2±0.3**
	39	6.1±0.1	6.1±0.2	6.0±0.2	5.9±0.1	6.2±0.3	6.7±0.7*
	52	5.8±0.2	5.8±0.3	6.2±0.2	5.8±0.1	5.8±0.4	6.3±0.4*
Albumin to globulin ratio	52	1.62±0.21	1.30±20*	1.12±0.15**	1.8±0.16	1.38±0.08*	1.39±0.21*
β-Globulin (g/dL)	39	1.2±0.1	1.1±0.2	1.2±0.1	1.0±0.10	1.1±0.1	1.4±0.1**
	52	1.0±0.1	1.2±0.2	1.4±0.1**	1.0±0.0	1.1±0.1	1.2±0.1**
γ-Globulin (g/dL)	27	0.5±0.0	0.5±0.1	0.5±0.0	0.4±0.0	0.4±0.1	0.7±0.3*
	52	0.4±0.1	0.6±0.2*	0.7±0.1**	0.4±0.0	0.5±0.1	0.7.±0.4

[a] ( )—Number of animals.

[b] Values for 39 and 52 weeks included only four females.

*Significantly different from control value ($p < 0.05$).

**Significantly different from control value ($p < 0.01$).

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and their ratios in the mid- and high-dose animals were increased. However, no dose- or time-related trend was noticed. In addition, many other biochemical parameters (levels of urea nitrogen, blood glucose, creatinine, sodium, calcium, chloride, and α_1- and α_2-globulins) showed significant changes in various dosed groups at sporadic measurement intervals. However, in most instances, either the change was not compound related or the reported values were within the range of biological variation.

The high-dose male that was sacrificed moribund at week 7 had increases in serum enzymes, glucose, total nitrogen and bilirubin, cholesterol, γ-globulin and abnormal serum electrolyte values when compared to controls or the reference range. High-dose female No. 4731, which was removed from the test diet at week 38, had similar abnormalities in clinical chemistry parameters prior to week 38; most of these changes returned to normal after 6 weeks of recovery, but total cholesterol remained lower than normal.

7. Urinalyses: Urine samples were collected from all animals of all treatment groups 2 weeks prior to study initiation and in study weeks 12, 27, 39, and 52. Samples were also collected from three animals (control male, low-dose male, mid-dose female) 1 week prior to study initiation and from a high-dose male (No. 4744) 1 week prior to study initiation and in week 6. The CHECKED (X) parameters were examined.

	Appearance†	X	Glucose†
	Volume†	X	Ketones†
X	Specific gravity†		Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†		Nitrate
X	Protein†	X	Urobilinogen
X	Leukocytes	X	Bacteria
X	Crystals	X	Sperm
X	Epithelial cells	X	Erythrocytes

Results: No compound-related intergroup differences in any of the determined parameters were observed.

8. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and all the CHECKED (X) tissues were collected for histological examination in the controls and high-dose animals. In the low- and mid-dose animals, only liver, kidney, and spleen were microscopically examined. In addition, sections of liver, kidney, and spleen from all the dosed animals were stained for identification of iron deposits. The organs marked XX were also

†Recommended by USEPA Subdivision F (October 1982) Guidelines.

weighed. High-dose female No. 4731, which was removed from the main study, was necropsied 4 months later than the scheduled date of sacrifice.

	Digestive system
	Tongue
X	Salivary glands†
X	Esophagus†
X	Stomach†
X	Duodenum†
X	Jejunum†
X	Ileum†
X	Cecum†
X	Colon†
X	Rectum†
XX	Liver†
X	Gall bladder†
X	Pancreas†
	Respiratory
X	Trachea†
X	Lung†

	Cardiovasc./Hemat.
X	Aorta thoracalis†
X	Vena cava caudalis
XX	Heart†
X	Bone marrow†
XX	Lymph nodes† (iliac, mesenteric)
XX	Spleen†
X	Thymus†
	Urogenital
XX	Kidneys†
X	Urinary bladder†
XX	Testes†
X	Epididymides
X	Prostate
	Seminal vesicle
XX	Ovaries
X	Uterus†

	Neurologic
XX	Brain† (cerebrum, cerebellum, medulla oblongata)
X	Peripheral nerve†
X	Spinal cord (cervical, lumber)†
XX	Pituitary†
X	Eyes (optic nerve)†
	Glandular
XX	Adrenals†
	Lacrimal gland
X	Mammary gland†
	Parathyroids†
XX	Thyroids†
	Other
X	Bone (rib)†
X	Skeletal muscle† (gastrocnemius)
X	Skin
	All gross lesions and masses

Results:

a. Organ Weights: Mean absolute weights for liver, spleen, and lymph nodes in mid-dose males were increased by 19, 22, and 48 percent, respectively. The corresponding increases in relative weights were 24, 27, and 49 percent (Table 4). Mean absolute weights for liver, spleen, and lymph nodes in high-dose males were increased by 14, 123, and 116 percent, respectively, with the corresponding relative weights increasing by 19, 107, and 117 percent. Mean absolute and relative weights for spleen in mid-dose females were increased by 21 and 22 percent and in high-dose females by 167 and 132 percent, respectively. In high-dose females, absolute and relative weights for liver (29 and 12 percent) and lymph nodes (54 and 31 percent) were also increased. However, due to the small number of animals evaluated, these increases in organ weights were found to be statistically insignificant.

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†Recommended by USEPA Subdivision F (October 1982) Guidelines.

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TABLE 4. Mean Absolute (g±SD) and Relative (g/10 kg body weight±SD) Organ Weights of Dogs Fed SN 49 537 for 52 Weeks[a]

Organ	Males/Dose (ppm)						Females/Dose (ppm)					
	0		300		1000		0		300		1000	
	Abs.[b]	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
Liver	337.6 ±59.9	352.5 ±20.8	400.5 ±51.8	438.9 ±116.1	385.2 ±77.3	421.2 ±68.3	316.1 ±75.9	356.2 ±30.4	309.8 ±71.2	351.4 ±30.7	399.7 ±112.7	394.4 ±68.0
Spleen	35.5 ±7.2	38.8 ±11.8	43.2 ±20.7	49.2 ±37.1	79.1 ±65.4	80.2 ±63.8	29.6 ±4.9	34.5 ±8.7	35.7 ±15.5	42.2 ±23.8	79.1 ±65.4	80.2 ±63.8
Lymph nodes	0.50 ±0.21	0.53 ±0.22	0.74 ±0.13	0.79 ±0.11	1.08 ±0.46	1.15 ±0.35	0.59 ±0.16	0.67 ±0.14	0.51 ±0.26	0.59 ±0.26	0.91 ±0.35	0.88 ±0.16

[a]N = five in 0- and 300-ppm groups and four in 1000-ppm groups.

[b]Abbreviations used:
Abs. -absolute
Rel.--relative.

b. Gross Pathology: According to the study authors, compound-related macroscopic lesions, summarized below, occurred only in males:

Mid-dose male No. 4702--red-brown content in the gall-bladder and small volume of water-clear fluid in the cephalic sinuses.

High-dose male No. 4707--small volume of water-clear fluid in the abdominal cavity.

High-dose male No. 4726--enlarged spleen.

High-dose male No. 4744--enlarged spleen, marked anemia, slight jaundice, and black-brown discoloration of the liver. This animal was sacrificed moribund in week 7.

The most commonly observed nonspecific lesions, reddening of the ureter mucosa and neck (six males, eight females) and prominent medullary rays in the kidneys (one male, five females) were randomly distributed in all the groups, though more prevalent in females. In addition, lesions, such as foci in the liver, cecum, and adrenal gland; cysts in the lung and ovary; pale thyroid glands; and reduced or discolored lymph nodes; occurred randomly in one or two males and/or females and were not considered toxicologically important.

c. Microscopic Pathology

1. Nonneoplastic: According to the study authors, no major histopathologic lesions of the kidney, liver, or spleen caused by SN 49 537 administration were observed in the low-dose animals. Compound-related lesions in the liver, kidney, and spleen of mid- and high-dose animals included hepatocellular dystrophy, increased Kupffer cells, and hemosiderosis. The term hemosiderosis is somewhat of a misnomer since iron positive pigment (Prussian blue staining) was positive only in the cases of "marked" degree of the finding (Table 5). The increase in hemosiderin (marked) deposits in the liver of mid-dose males and high-dose males and females was primarily in those animals where severe effects on red cell parameters were found. In high-dose male No. 4744 (sacrificed moribund) there was also increased hemosiderin in the liver and hematopoiesis in the spleen and kidney. The two affected mid-dose males had iron-negative pigment in the kidney and spleen. The two high-dose females with severe anemia, in addition to marked hemosiderin in the liver, had increased Kupffer cells, hemosiderin in the spleen, increased splenic hematopoiesis, and iron-negative

TABLE 5. Summary of Histopathological Observations in Dogs Sacrificed Moribund and after 52 Weeks[a]

Organ/Observation	Dose (ppm)							
	Control		100		300		1000	
	M	F	M	F	M	F	M	F
Liver								
Hepatocellular dystrophy or necrosis	0	0	0	0	0	0	1	1
Increase of Kupffer cells	0	1	0	1	3	0	5	3
Hemosiderosis in Kupffer cells and/or intralobular connective tissue								
--minimal to slight	2	2	4	4	2	4	1	2
--moderate	0	0	0	0	0	0	1	0
--marked	0	0	0	0	2	0	2	2
Intra-/interlobular cell infiltration with lymphocytes	2	4	3	2	4	2	5	5
Kidney								
Accumulation of a brown pigment in the tubular epithelia of the renal cortex								
--iron negative	0	0	0	0	4	1	4[b]	4
--iron positive (hemosiderin)	0	0	0	0	2	0	2[b]	1
Calcified casts in the collecting tubuli of the renal medulla	5	5	5	5	2	5	5	4
Spleen								
Hemosiderosis								
--minimal to slight	5	5	4	3	2	2	2	0
--moderate	0	0	1	2	2	3	2	3
--marked	0	0	0	0	1	0	1	2
Moderate to marked congestive hyperemia	4	5	2	4	1	2	3	2

(Continued)

[a] Five tissues were examined for each organ. One high-dose male was sacrificed moribund in week 7. One high-dose female was removed from the study in week 38 and sacrificed 33 weeks thereafter.

[b] Cortex for one high-dose male was not included in the examination.

[c] One animal had [illegible]

TABLE 5. Summary of Histopathological Observations in Dogs Sacrificed Moribund and after 52 Weeks[a] (continued)

Organ/Observation	Dose (ppm)							
	Control		100		300		1000	
	M	F	M	F	M	F	M	F
Lung								
Interstitial pneumonia (diffused or lobular)								
--slight	4	5	No Data				1	4
--moderate	1	0					4	1
Alveolar emphysema	5	2					0	1
Thyroid								
Interfollicular cell increase	3	0	No Data				3	3
Thymus								
Early or progressive involution	1	0	No Data				4	0
Stomach								
Localized increased accumulation of lymphocytes in the mucosa	3	2	No Data				4	3
Small Intestine								
Increased accumulation of lymphocytes in the mucosa	1	1	No Data				1	0

(Continued)

[a] Five tissues were examined for each organ. One high-dose male was sacrificed moribund in week 7. One high-dose female was removed from the study in week 38 and sacrificed 33 weeks thereafter.

[b] Cortex for one high-dose male was not included in the examination.

[c] One animal had lesions on both sites.

TABLE 5. Summary of Histopathological Observations in Dogs Sacrificed Moribund and after 52 Weeks[a] (concluded)

Organ/Observation	Dose (ppm)							
	Control		100		300		1000	
	M	F	M	F	M	F	M	F
Large Intestine								
Predominantly localized increased infiltration of lymphocytes	4	3	No Data				5	5
Gallbladder								
Localized infiltration of lymphocytes in the mucosa	2	1	No Data				5	5
Lymph Nodes (2 sites)								
Hemosiderosis in the sinus	1	0	No Data				1	2[c]
Cell increase in the sinus	1	0					0	0
Atrophic lymphadenitis	0	1					0	0
Follicular hyperplasia	0	1					0	2[c]

[a]Five tissues were examined for each organ. One high-dose male was sacrificed moribund in week 7. One high-dose female was removed from the study in week 38 and sacrificed 33 weeks thereafter.

[b]Cortex for one high-dose male was not included in the examination.

[c]One animal had lesions on both sites.

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pigment in the tubular epithelium of the kidney. Localized infiltration of lymphocytes in the mucosa of the gallbladder was found in all high-dose animals as compared to two of five control males and one control female. Early or progressive involution in the thymus was found in four of five of the high-dose females compared to one of five in the control group, and interfollicular cell increase in the thyroid of high-dose females (3/5) was also reported. Follicular hyperplasia (control, 1/5; high-dose, 2/5) in the lymph nodes of high-dose females showed a 20 percent increase. In high-dose female No. 4731, which was removed from the main study in week 38 for recovery and sacrificed 33 weeks thereafter, the only major lesions present were marked hemosiderosis in the liver and spleen.

Interstitial pneumonia was found in all control and high-dose animals; lungs of low- and mid-dose animals were not examined. However, the severity of this finding was slight in four of five control males but moderate in four of five high-dose males.

2. Neoplastic: No neoplastic lesions were observed.

D. STUDY AUTHORS' CONCLUSIONS: The study authors concluded that administration of 100 ppm SN 49 537 did not produce toxicity in the dogs; therefore, this concentration (the lowest dose administered) was proposed as the NOEL. SN 49 537 induced hemolytic anemia in a few animals at 300- and 1000-ppm levels of feeding, leading to some clinical signs, changes in hematologic and biochemical parameters, and histopathologic lesions in liver, kidney, and spleen. Hematologic changes due to anemia, and mostly confined to a few animals, showed a tendency to normalize during the treatment and were reversible after withdrawal of the test compound (one 1000-ppm female). The study authors concluded that there was individual animal susceptibility to the test compound that resulted in the induction of anemia.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS: This study is unacceptable since histopathologic examination was not conducted on all tissues in the mid- and low-dose groups. This is a Guideline requirement in chronic non-rodent studies. In the present study, only sections of liver, kidney, and spleen were stained for histologic examination in the mid- and low-dose groups.

There were severe weight losses in one high-dose male which resulted in its moribund sacrifice (week 7) and in one high-dose female which was removed from the study at week 38 to permit recovery. There was a severe anemic response in both of these animals. After removal of these animals from the study, however, there were still at least four animals/sex/group.

It is our assessment that the anemic response was compound related. There was no effect on hematologic parameters at 100 ppm. There may be a predisposition of some dogs to the anemic response, since the hematology values for the unaffected dogs in the mid- and high-dose groups were completely within the normal range; however, in the affected dogs the red cell parameter effects were dramatic.

Statistical analyses of the hematology data were inadequate. Analysis of variance should have been performed to test for homogeneity before using Dunnett's test. Any statistical evaluation of these data would be weak because of the small sample size and wide individual variation in values. In our opinion it is more reasonable to show the abnormal values (as in Table 1 of this report) than to present mean values. Survival of a dog with a hemoglobin (Hb) value of 1.7 g/dL (female No. 4731) is suspect since normal Hb values are about 15 g/dL.

In some mid- and high-dose dogs, there was correlation between marked hemosiderosis in liver and spleen and the presence of anemia. The minimal hemosiderosis in the livers of low-dose males and females is not considered of toxicologic importance. This is supported by the absence and iron-positive reaction with histologic Prussian blue staining.

Significant ($p < 0.05$ or 0.01) compound-related changes in clinical chemistry parameters were observed in the high-dose and to a lesser extent in the mid-dose animals. These included increases in alkaline phosphatase, total serum protein and globulins, and a reduction in serum albumin. However, we do not assess that any of the observed changes were biologically important because there were no clear dose-related trends.

Mean absolute and relative weights for spleen, liver, and lymph nodes were substantially increased in mid- and high-dose males. Some high-dose males had enlarged spleens. Absolute and relative weights for spleen were increased in the mid- and high-dose females and for liver and lymph nodes in the high-dose females. These changes were statistically insignificant (because of the small number of animals used in the test).

The reviewers could not assess the effect of intercurrent infection in the lungs and the associated histopathologic finding of interstitial pneumonia on the conduct of the study. The degree of the lesion was slight in all control females and in four of five control males. A dose-response effect could not be evaluated because there was a lack of histology of the lungs in low- and mid-dose groups. However, there was no impact on body weight gain or on leukocyte parameters, except in one high-dose male that was sacrificed moribund and one high-dose female removed from dosing. Therefore, we cannot conclude that pneumonia confounded the results.

Under the conditions of the study the LOAEL is 300 ppm based primarily on anemia and the NOAEL is 100 ppm.

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APPENDIX A

Materials and Methods

Thidiazuron toxicology review

Page _____ is not included in this copy.

Pages 26 through 34 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

006330

EPA: 68-02-4225
DYNAMAC No. 255-8
July 10, 1987

DATA EVALUATION RECORD

THIDIAZURON

Mutagenicity--*In vitro* Cytogenetic Study with Human Lymphocytes

STUDY IDENTIFICATION: Ivett, J. L., and Galloway, S. M. Mutagenicity evaluation of thidiazuron, batch No. 7/9.82 in an *in vitro* cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures. (Unpublished study No. LBI 20990 prepared by Litton Bionetics, Inc., Kensington, MD, for Schering AG, Berlin, FRG; dated June 1984.) Accession No. 262816.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-9-87

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006330

1. CHEMICAL: Thidiazuron.

2. TEST MATERIAL: Thidiazuron from batch No. 7/9.82 was described as an off-white powder with a purity of 98.9%.

3. STUDY/ACTION TYPE: Mutagenicity--In vitro cytogenetic study with human lymphocytes.

4. STUDY IDENTIFICATION: Ivett, J. L., and Galloway, S. M. Mutagenicity evaluation of thidiazuron, batch No. 7/9.82 in an in vitro cytogenetic assay measuring chromosome aberration frequencies in human lymphocytes from whole blood cultures. (Unpublished study No. LBI 20990 prepared by Litton Bionetics, Inc., Kensington, MD, for Schering AG, Berlin, FRG; dated June 1984.) Accession No. 262816.

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7. CONCLUSIONS:

A. Thidiazuron was tested in an in vitro human lymphocyte cytogenetic assay under nonactivated and S9-activated conditions. The nonactivated assay, conducted with 2.5, 5.0, 10.0, 20.0, and 40.0 µg/mL thidiazuron, produced equivocal negative results because of the borderline activity of the positive control, mitomycin C (Mit C) at 0.25 µg/mL (results for the 0.2 µg/mL level were not reported), and the presence of rare complex chromosome aberrations at two assayed doses of the test material (5 and 20 µg/mL).

In the presence of S9 activation, five doses (25 to 300 µg/mL) did not induce a clastogenic response; the test material was cytotoxic at 300 µg/mL. The positive control, cyclophosphamide (CP) at 120 µg/mL/+S9, induced a significant ($p < 0.01$) clastogenic response, indicating that the S9-activated system had an adequate level of sensitivity. We conclude, therefore, that in the presence of S9 activation, thidiazuron was assayed up to a cytotoxic level with no effect.

B. The nonactivated assay was unacceptable because the sensitivity of the system for detecting a direct-acting clastogen is questionable and rare complex aberrations were seen at two assayed doses of the test material. However, the S9-activated assay was acceptable.

8. RECOMMENDATIONS:

It is recommended that the nonactivated assay be repeated with an appropriate level of the positive control and with a human lymphocyte system that has a demonstrated ability to detect clastogenic responses. It is also recommended that either separate experiments with lymphocytes from different donors or replicate cultures from different donors be included in the study.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material:

a. Description: Thidiazuron from batch No. 7/9.82 was described as an off-white powder with a purity of 98.9%.

---

[1]Only items appropriate to this DER have been included.

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b. Solubility Determinations: Solubility of thidiazuron in dimethylsulfoxide (DMSO) was determined to be 60 mg/mL. However, concentrations of the test material ≥50 mg/mL precipitated in culture medium.

Based on the solubility of the test material, a stock solution of 50 mg/mL was prepared in DMSO to yield test concentrations ranging from 0.017 to 500 µg/mL.

2. Cell Line: Human lymphocytes were obtained from the venous blood of a single donor; no information regarding the donor was provided. Cultures were initiated in RPMI 1640 medium supplemented with 15% fetal calf serum (FCS), 1% phytohemagglutin (PHA-M), and antibiotics. Cultures were grown for approximately 24 hours at 37°C without treatment to permit PHA stimulation.

3. S9 Fraction: The S9 fraction, which was obtained from an unspecified commercial source, was derived from the livers of rats induced with Aroclor 1254. The S9 reaction mixture contained 15 µL/mL rat liver S9.

4. Preliminary Cytotoxicity Assay: Single cultures of PHA-stimulated lymphocytes (0.3 mL whole blood grown in 4.7 mL of complete medium containing 25 µM BrdU) were exposed to half-log dilutions of the test material ranging from 0.017 to 500 µg/mL, the solvent control (DMSO), or the positive controls [ethylmethanesulfonate (EMS) at 0.125 µL/mL/-S9; Cyclophosphamide (CP) at 7.5 µg/mL/+S9] with or without S9 activation.

In the nonactivated system, cells were exposed for approximately 50 hours to the appropriate test material doses and negative, solvent, or positive controls. Cultures were washed, resuspended in medium containing 0.1 µg/mL colcemid, and incubated for an additional 2 hours. In the S9-activated system, cultures were centrifuged after the initial 24-hour incubation/stimulation period, resuspended in medium without FCS, and exposed for 1 hour to the test material or controls in the presence of the S9 reaction mixture. After exposure, cells were washed twice, resuspended in fresh medium, and incubated for approximately 50 hours. Colcemid was added 2 hours prior to harvest.

After incubation, metaphase cells were collected, treated with a hypotonic 0.075 M solution of KCl, and washed three times in fixative (methanol:acetic acid, 3:1); slides were prepared. Estimation of cell-cycle delay was accomplished by staining the cells with the modified fluorescent-plus-Giemsa

techniques of Perry and Wolff[2] and Goto et al.[3] One hundred metaphase cells per group were examined for the percentage of first (M_1), second (M_2), and third (M_3) division metaphase cells.

5. Cytogenetic Assay:

   a. Treatment: Cultures prepared in duplicate (0.6 mL of whole blood grown in 10 mL of complete medium) were exposed to test material doses ranging from 2.5 to 100 µg/mL/-S9 and 50 to 500 µg/mL/+S9. Controls included media alone, the solvent (DMSO), and Mit C at 0.25 µg/mL (-S9) or CP at 50 µg/mL (+S9).

   The assay was conducted as described for the preliminary cytotoxic test; however, BrdU was not included in the medium and metaphase cells were stained with 5% Giemsa.

   b. Metaphase Analysis: All slides except the positive controls were coded prior to scoring. A maximum of 100 cells (50 cells/replicate culture) were scored per dose level.

6. Statistical Methods: The data were evaluated for statistical significance at p values of <0.05 and <0.01 by Fisher's exact test. The negative (culture medium) and solvent controls were pooled if no statistical differences were calculated in the Fisher's exact test.

7. Evaluation Criteria: The test material was considered clastogenic if evidence of increasing amounts of damage coincided with increasing doses of the test material.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: The cytotoxicity assay was conducted with test doses ranging from 0.017 to 500 µg/mL, separated by half-log dilutions, in the presence or absence of S9 activation.

   1. Without S9 Activation: Heavy compound precipitation was reported at 500 µg/mL and the results for this dose, which

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[2]Perry, P. and Wolff, S. New Giemsa method for the differential staining of sister chromatids. Nature 251: 156-158 (1974).

[3]Goto, K., Maeda, S., Kano, Y., and Sugiyama, T. Factors involved in differential Giemsa-staining of sister chromatids. Chromosoma 66: 351-359 (1978).

are similar to the negative control values, suggest that the insoluble test material did not interact with the lymphocytes (Table 1). This conclusion is supported by the cytotoxic effects that were observed at the lower doses where precipitation did not occur (50 and 167 µg/mL). For the remaining scored doses (1.7, 5.0, and 16.7 µg/mL), a slight delay in cell-cycle progression was observed. Based on these results, the nonactivated assay was performed with doses ranging from 2.5 to 100 µg/mL with a normal 50-hour cell harvest.

2. With S9 Activation: As with the nonactivated assay, compound precipitation was also observed at the highest S9-activated dose. However, there was a slight cell-cycle delay, which the authors interpreted as valid evidence of compound-target cell interaction. At the only other scored S9-activated dose (167 µg/mL), the percentage of M_1, M_2, and M_3 cells was comparable to the control value. Based on these data, doses ranging from 50 to 500 µg/mL were tested in the S9-activated cytogenetic assay with a normal 50-hour harvest time. Representative results are presented in Table 1.

B. Cytogenetic Assay:

1. Nonactivated Test Material: At the 40-µg/mL nonactivated dose, a reduction in the mitotic index and recovered metaphase cells was reported. No results were obtained at higher levels. Results from evaluated doses (2.5, 5.0, 10.0, 20.0, and 40.0 µg/mL) showed no significant increase in chromosome aberrations; however, single quadriradials, which are rare complex chromosome aberrations, were observed in metaphase cells exposed to 5 and 20 µg/mL of the test material. It should be noted that although the positive control (Mit C at 0.25 µg/mL) induced a significant increase ($p < 0.05$) in the number of aberrations per cell, the total number of aberrations scored in 50 metaphase cells was four (two quadriradials and two chromosome breaks).

   Representative results are shown in Table 2.

2. S9-Activated Test Material: Due to technical problems including unexpected compound cytotoxicity, unusual compound precipitation, and no response with the positive control, three S9-activated assays were performed. Although the authors reported the results of the second trial, they were considered invalid by our reviewers because the positive control (CP at 50 µg/mL) did not induce a clastogenic response. However, data from the second trial indicated that the test material was cytotoxic at 400 µg/mL. For the third trial, five doses ranging from 25 to 300 µg/mL were assayed and the concentration of the positive control was increased to 120 µg/mL.

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TABLE 1. Representative Results from the Preliminary Test for Delay of Cell-Cycle Progression with Thidiazuron

Substance	Dose/ mL	S9 Activation	% Cells[a] M_1	M_2	M_3
Negative Control					
Culture medium	--	-	12	42	46
	--	+	33	50	17
Solvent Control					
Dimethylsulfoxide	10 µL	-	16	53	31
	10 µL	+	9	41	50
Positive Control					
Ethylmethanesulfonate	0.125 µL	-	24	51	25
Cyclophosphamide	7.5 µg	+	15	42	43
Test Material					
Thidiazuron	16.7 µg[b]	-	46	48	6
	50.0 µg	-	____	Toxic	____
	167.0 µg	-	____	Toxic	____
	500.0 µg[c]	-	20	43	37
	167.0 µg[d]	+	18	35	47
	500.0 µg[c]	+	40	49	11

[a] Percent cells in first (M_1), second (M_2), or third (M_3) cell division.

[b] Results for lower scored doses (1.7 and 5.0 µg/mL) showed a similar trend of slight cell-cycle delay.

[c] Compound precipitation.

[d] Lowest evaluated dose.

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TABLE 2. Representative Results of the Human Lymphocyte in vitro Cytogenetic Assay with Thidiazuron Following a 50-Hour Cell Harvest

Substance	Dose (µg/mL)	S9 Activation	No. of Cells Scored	No. of Aberrations per Cell	% Cells with Aberrations	% Cells with >1 Aberrations
Pooled Negative Control						
Medium and dimethylsulfoxide	—	-	100	0.01	1.0	0.0
	—	+[a]	100	0.02	2.0	0.0
Positive Control						
Mitomycin C	0.25	-	50	0.10*	8.0	2.0
Cyclophosphamide	120.0	+[a]	25	0.48**	24.0	12.0
Test Material						
Thidiazuron	20.0[b]	-	100	0.03	2.0	1.0
	40.0[c]	-	56	0.00	0.0	0.0
	200.0[d]	+[a]	100	0.01	1.0	0.0

[a]Results from the third S9-activated trial only; due to technical difficulties, the S9-activated assay was performed three times.

[b]Highest noncytotoxic dose without S9 activation. Results for lower doses (2.5, 5, and 10 µg/mL) showed no appreciable increase in aberrations; however, single quadriradials were observed at 5 and 20 µg/mL.

[c]Reduced metaphase recovery and mitotic index (indicative of cytotoxicity).

[d]Highest noncytotoxic S9-activated dose. Results for lower doses (25, 50, and 100 µg/mL) were comparable or lower than the pooled negative control values.

*Significantly higher ($p < 0.05$) than the pooled negative control, determined by Fisher's exact test.

**Significantly higher ($p < 0.01$) than the pooled negative control, determined by Fisher's exact test.

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In contrast to the cytotoxicity data presented for the second trial (cytotoxic at 400 µg/mL), complete cytotoxicity (no metaphase cells recovered) was achieved at 300 µg/mL in the third assay. No increase in aberrations per cell, percent cells with aberrations, or percent cells with >1 aberration was scored at any of the noncytotoxic doses. The positive control induced marked increases in all measured parameters; the increase in aberrations per cell was significant ($p < 0.01$).

Representative results are presented in Table 2.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded, "Thidiazuron was negative in the aberration test under the conditions of these assays."

B. A quality assurance statement was signed and dated March 13, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the results reported for the nonactivated cytogenetic study with thidiazuron do not support the study authors' conclusions. The borderline response of the positive control (Mit-C) and the presence of rare chromosome aberrations at two nonactivated doses should have been sufficient reason to repeat the nonactivated study. The induction of quadriradials generally suggests a clastogenic response; hence, their presence at test doses in conjunction with a weak response for the positive control raises doubts regarding the sensitivity of the peripheral lymphocytes used in the nonactivated assay to detect a weak clastogenic effect; this portion of the assay is, therefore, unacceptable.

Although the study authors had some difficulties in establishing the lowest cytotoxic dose of S9-activated thidiazuron, this was probably due to the solubility properties of the test material rather than technical error. The findings from the third trial indicate that S9-activated thidiazuron was assayed up to a cytotoxic dose with no evidence of a clastogenic effect. The results with the positive control (CP at 120 µg/mL/+S9) further suggest that an appropriate level of assay sensitivity to detect clastogenesis was achieved. We assess, therefore, that the S9-activated human lymphocyte assay with thidiazuron was acceptable.

It is noted, however, that although not required, it is strongly recommended that *in vitro* human lymphocyte cytogenetic assays should be performed with lymphocytes collected from different donors (i.e., each culture at each experimental point should be from separate donors or the entire experiment should be repeated with new donor lymphocytes).

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Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI p. 2; Appendix B, Protocol, CBI pp. 12-19.

APPENDIX A

Materials and Methods

Thidiazuron toxicology review

Page _____ is not included in this copy.

Pages 46 through 55 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

_X_ FIFRA registration data

___ The document is a duplicate of page(s) _____

___ The document is not responsive to the request

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

DYNAMAC CORPORATION

006330

Dynamac Corporation
The Dynamac Building
11140 Rockville Pike
Rockville, Maryland 20852
Telephone 301-468-2500
Telex: 248838

July 10, 1987

Ms. Caroline Gordon
Hazard Evaluation Division - CM - Room 720
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Ms. Gordon:

Enclosed is a final report for the following DER - THIDIAZURON:

- Mutagenicity--In vitro Cytogenetic Study with Human Lymphocytes - Study No. LBI 20990. Accession No. 262816. Dynamac No. 255-B. EPA No. 2-55.

We have enclosed the confidential business information for the above report.

Sincerely,

Robert J. Weir, Ph.D.
Program Manager

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EPA: 68-02-4225
DYNAMAC No. 255-C
July 10, 1987

DATA EVALUATION RECORD

THIDIAZURON

Chronic Toxicity and Oncogenicity Study in Rats

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 7-10-87

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EPA: 68-02-4225
DYNAMAC No. 255-C
July 10, 1987

DATA EVALUATION RECORD

THIDIAZURON

Chronic Toxicity and Oncogenicity Study in Rats

REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Patricia Turck
Date: July 10, 1987

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

Signature: William L. McLellan
Date: July 10, 1987

APPROVED BY:

I. Cecil Felkner, Ph.D.
Chronic Toxicity/Oncogenicity
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 7-16-87

Stephen C. Dapson, Ph.D.
EPA Reviewer, Section V
(TS-769C)

Signature: Stephen C. Dapson
Date: 9/21/87

Quang Q. Bui, Ph.D., D.A.B.T.
Acting EPA Section Head
Section V (TS-769C)

Signature: Quang Q. Bui
Date: 9-22-87

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DATA EVALUATION REPORT

TOX. CHEM. NO.:
MRID NO.:

STUDY TYPE: Chronic toxicity and oncogenicity study.

ACCESSION NUMBER: PF 82.801, 262816-262819.

TEST MATERIAL: Thidiazuron, technical grade (SN 49537).

SYNONYM(S): N-phenyl-N'-(1,2,3-thiadiazol-5-yl)urea.

STUDY NUMBER(S): RCC Project 011924.

SPONSOR: Schering AG, Berlin, Federal Republic of Germany.

TESTING FACILITY: RCC, Research and Consulting Company, AG, Itingen, Switzerland.

TITLE OF REPORT: 104-Week Chronic Toxicity and Oncogenicity Study with Thidiazuron Technical in the Rat.

AUTHOR(S): Sachsse, K.

REPORT ISSUED: April 15, 1986.

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CONCLUSIONS:

When thidiazuron was fed to Wistar (KFM-Han) rats at dietary levels of 0, 70, 200, or 600 ppm, there were no overt signs of toxicity or dose-related effects on mortality, pharmacotoxic signs, clinical laboratory findings, absolute or relative organ weights, and oncogenic changes at any site. Slight, sometimes significant decreases in body weight and food consumption were noted for 600-ppm males when compared to controls. No changes in body weight or food consumption were noted for females at any dose level. The LOEL for male rats, based on minimal changes in body weight and food consumption, is 600 ppm (35.9 mg/kg/day). The NOEL for systemic toxicity is 200 ppm (11.8 mg/kg/day) and 600 ppm of thidiazuron in the diet for males and females, respectively. The Maximum Tolerated Dose (MTD) for thidiazuron was not achieved.

Classification: Core Minimum for chronic toxicity. Individual or summary data for ophthalmologic examinations and for clinical observations were not provided. Further testing for oncogenicity is required because the MTD was not achieved. This study is considered supplementary data for oncogenicity.

A. MATERIALS:

1. Test Compound: Thidiazuron technical (SN 49537); description: beige powder, batch No. 7/9.82; purity: >98.7%.

2. Test Animals: Species: rat; strain: Wistar KFM-Han; age: approximately 5 weeks at initiation; weight: males--53-86 g and females--36-67 g; source: KFM Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf/Switzerland.

B. STUDY DESIGN:

1. Animal Assignment: After at least a 1-week acclimation period, animals were assigned to the following test groups using a random algorithm:

Test group	Dose in diet (ppm)	Main study (24 months)		Interim sacrifice (12 months)	
		Male	Female	Male	Female
1 Control	0	60	60	10	10
2 Low (LDT)	70	60	60	10	10
3 Mid (MDT)	200	60	60	10	10
4 High (HDT)	600	60	60	10	10

In addition, 20 animals/sex were used for pretest studies.

Dose levels were chosen based on a previous long-term feeding study conducted on Charles River CD rats (conducted by IBT).

Rats were individually housed in a barrier system and received food (Kliba rat diet) and water ad libitum. Diet and water were analyzed for contaminants.

2. Diet Preparation: Diet was prepared bimonthly by mixing the test material with microgranulated feed and then pelleting the mixture. The diet was stored at room temperature in disposable paper bags. Samples of treated food were analyzed for stability before the start of the study and at week 98 and for concentration and homogeneity at weeks 0, 6, 11, 14, 20, 25, 27, 32, 38, 42, 45, 50, 56, 58, 66, 67, 74, 78, 86, 90, 94, 98, and 102.

   Results: Thidiazuron technical in rodent feed was found to be homogeneous and was stable for 21 days at room temperature. Table 1 summarizes the results of diet analyses. Levels of thidiazuron technical in the diet were found to range from 73.5 to 117.6% of target values. Mean percent concentrations of target levels were 91±8.6, 93±7.7, and 95±7.9% for the 70-, 200-, and 600-ppm dose groups, respectively. Values obtained for the majority of mixings were within acceptable limits.

4. Statistics: Body weights, food consumption, organ weights, and clinical laboratory data were analyzed using a univariate one-way analysis of variance and either the Dunnett or Steel test. For overall unscheduled mortality data, Fisher's exact test was used.

5. A quality assurance statement was signed and dated April 10, 1986.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected twice daily for signs of toxicity and mortality. In addition, all animals were given a weekly physical examination for palpation of masses. Any abnormalities were recorded.

   Results: The author reported observing no local or systemic toxicity that could be attributed to test material administration. However, no individual or summary data on pharmacotoxic observations or results of weekly palpations were presented.

   Representative mortality and survival data are presented in Table 2. A slight (nonsignificant) increase in mortality was reported for the 600-ppm dosed females when compared to controls. No other differences in mortality were noted among treated and control groups.

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TABLE 1. Representative Dietary Analysis of Thidiazuron, as Percent of Targeted Dose (±S.D.)[a], Fed to Rats for 2 Years

Targeted Dietary Level (ppm)	Percent of Target at Week		
	0	56	102
70	92±5.6	88± 7.0	93±1.0
200	105±4.5	98±10.3	98±5.3
600	117±2.9	90± 7.6	99±1.9

[a]Values were obtained from the mean of three samples taken from the top, middle, and bottom of the bag of feed.

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TABLE 2. Representative Results of Mortality (Percent Survival) of Male and Female Rats Fed Thidiazuron Technical for 2 Years

Dietary Level (ppm)	Cumulative Mortality (Percent Survival) at Day		
	300[a] (0-300)	600[b] (301-600)	Study Termination[b] (600-751)
		Males	
0	1 (98.6)	2 (96.7)	11 (81.7)
70	2 (97.1)	5 (91.6)	10 (83.3)
200	3 (95.7)	7 (88.3)	13 (78.3)
600	0 (100)	4 (93.3)	9 (85.0)
		Females	
0	1 (96.6)	2 (88.3)	17 (71.7)
70	0 (100)	10 (83.3)	16 (73.3)
200	0 (100)	7 (88.3)	17 (71.7)
600	2 (97.1)	9 (85.0)	19 (68.3)

[a]Based on 70 rats/sex/group.
[b]Based on 60 rats/sex/group.

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2. Body Weight: Animals were weighed once weekly for 13 weeks and twice monthly thereafter.

   Results: Mean body weight data for selected intervals are presented in Table 3. The author reported slight reductions in mean body weight gains for 600-ppm dosed males. Slight, but statistically significant reductions in mean body weights of the 600-ppm dosed males were sporadically noted throughout the test period when compared to controls. In addition, consistent decreases at the 600-ppm level were noted in male rats from study weeks 73 through 89. No other changes in body weight were noted between treated and control groups.

3. Food Consumption and Compound Intake: Consumption was determined weekly for the first 13 weeks and twice monthly thereafter; from these values, the mean daily diet consumption was calculated. The nominal dose level was calculated from the food consumption and body weight data. Feed efficiency was not calculated.

   Results: Representative food consumption data are presented in Table 4. Slight, but statistically significant decreases in food consumption were noted for 600-ppm dosed males when compared to controls during the first year on study, and sporadic reductions were noted thereafter.

   The reported mean nominal daily thidiazuron technical consumption was 3.7, 10.6, and 31.7 mg/kg body weight for males and 4.6, 13, and 40.1 mg/kg body weight for females fed 70, 200, or 600 ppm, respectively.

4. Ophthalmology and Hearing: Ophthalmological and hearing examinations were performed prior to study initiation, after 3, 6, and 12 months, and at terminal sacrifice on 10 animals/sex/group. The hearing test was performed using a tone generator (frequency, 10 KHz; duration, 30 seconds). A positive reaction consisted of Preyer's reflex.

   Results: The author reported that ophthalmic and hearing examinations revealed no changes attributable to test material administration. However, individual and summary data were not presented.

5. Hematology and Clinical Chemistry: Blood was collected at 14, 26, 52, and 104 weeks of treatment for hematology and clinical analysis from 20 animals/sex/group and at week 78 from 10 animals/sex/group. Animals were fasted overnight (approximately 18 hours) prior to collection. Prior to treatment, blood was collected from 20 nonfasted animals/sex to monitor health status. Blood was collected from anesthetized animals via the retroorbital plexus. The CHECKED (X) parameters were examined.

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TABLE 3. Mean Body Weights (±S.D.) of Male and Female Rats Fed Thidiazuron Technical for 2 Years

Dietary Level (ppm)	Initial Mean Body Weight	Mean Body Weight (g) at Study Week				
		13	51	77	89	104
			Males			
0	123±10.9	388±30.0	522±52.6	579±67.4	594±75.3	584±79.0
70	122±10.7	395±37.8	534±59.7	574±67.1	583±61.9	582±67.8
200	119± 9.2*	396±32.9	527±50.9	574±67.7	597±77.6	603±71.3
600	119±10.3	387±34.9	502±51.4	550±57.1*	561±64.4*	560±59.6
			Females			
0	89± 9.1	220±21.0	286±38.4	313±52.7	327±57.2	339±65.5
70	88± 7.9	223±18.3	287±31.6	326±44.0	348±43.0	357±50.2
200	90± 7.2	224±17.4	290±34.7	329±45.0	342±48.0	355±52.3
600	89± 7.9	223±19.8	286±32.6	326±50.6	341±56.6	354±61.7

*Significantly different from control values ($p < 0.05$).

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TABLE 4. Mean Food Consumption (±S.D.) of Male and Female Rats Fed Thidiazuron Technical for 2 Years

Dietary Level (ppm)	Mean Food Consumption (g/animal/day) at Study Week					
	1	13	27	51	79	104
			Males			
0	19±2.3	22±2.0	23±2.2	23±2.7	24±3.7	23±4.4
70	18±1.7*	23±2.0	23±2.0	22±2.5**	24±2.6	23±2.4
200	18±1.9*	22±2.0	22±2.1	22±2.4**	23±4.3	24±3.5
600	17±1.5**	22±1.8	23±2.8	22±2.1**	23±2.7	23±3.9
			Females			
0	14±1.3	16±1.7	15±1.9	17±2.0	16±2.4	18±3.2
70	14±1.4	16±2.6	16±2.0	17±2.5	17±3.1	17±3.8
200	14±1.2	16±1.9	16±2.0	16±2.2*	17±2.6	18±3.7
600	14±1.3	16±1.7	17±3.2**	17±2.4	17±3.1	18±2.5

*Significantly different from control values ($p < 0.05$).

**Significantly different from control values ($p < 0.01$).

a. Hematology

X Hematocrit (HCT)†
X Hemoglobin (HGB)†
X Leukocyte count (WBC)†
X Erythrocyte count (RBC)†
X Platelet count†
X Reticulocyte count (RETIC)
X Red cell morphology
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
X Coagulation:thromboplastin time (PT)

Results: The author reported that no compound-related differences were noted in hematology parameters. The few statistically significant differences noted were considered of normal biological variance. Reference values for the testing laboratory for rats of the same strain, sex, and age were provided in support.

b. Clinical Chemistry

Electrolytes
X Calcium†
X Chloride†
Magnesium†
X Phosphorus†
X Potassium†
X Sodium†

Enzymes
X Alkaline phosphatase (ALP)
X Cholinesterase
X Creatinine phosphokinase†
X Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)†
X Serum aspartate aminotransferase (SGOT)†
X Gamma glutamyltransferase (GGT)
X Urea

Other
X Albumin†
X Albumin/globulin ratio
X Blood creatinine†
X Blood urea nitrogen†
X Cholesterol†
X Globulins
X Glucose†
X Total bilirubin†
X Total protein†
Triglycerides
X Direct bilirubin

Results: No differences that could be attributed to test material administration were reported. Mean values that differed significantly from control were found sporadically. However, their occurrence was infrequent, the values were within the normal range, and there was no dose- or time-related pattern.

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†Recommended by Subdivision F (October 1982) Guidelines.

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6. Urinalysis: Urine was collected from 20 fasted animals/sex/group at 14, 26, 52, and 104 weeks of treatment and from 10 fasted animals/sex/group at week 78 (these same animals were used for hematology and clinical chemistry parameters). The CHECKED (X) parameters were examined.

X	Appearance†	X	Glucose†
X	Volume†	X	Ketones†
X	Specific gravity†	X	Bilirubin†
X	pH	X	Blood†
X	Sediment (microscopic)†	X	Nitrate
X	Protein†	X	Urobilinogen

Results: No treatment-related effects were noted after review of urinalysis data..

7. Sacrifice and Pathology: All animals that died or were sacrificed on schedule were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs from all animals were also weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerve (sciatic nerve)†
X	Esophagus†	X	Bone marrow†		
X	Stomach†	X	Lymph nodes†	X	Spinal cord (3 levels)
X	Duodenum†	X	Spleen†	X	Pituitary†
X	Jejunum†	X	Thymus†	X	Eyes (optic nerve)†
X	Ileum†				
X	Cecum†		Urogenital		Glandular
X	Colon†	XX	Kidneys†	XX	Adrenals†
X	Rectum†	X	Urinary bladder†		Lacrimal gland
XX	Liver†	XX	Testes†	X	Mammary gland†
	Gall bladder†	X	Epididymides	X	Parathyroids†
X	Pancreas†	X	Prostate	X	Thyroids†
		X	Seminal vesicle	X	Harderian glands
	Respiratory	XX	Ovaries		
X	Trachea†	X	Uterus†		Other
X	Lung†			X	Bone (sternum)†
				X	Skeletal muscle†
				X	Skin
				X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines.

All of the above organs were examined histopathologically from all animals dying or sacrificed moribund prior to terminal sacrifice and from animals of the control and 600-ppm dosed groups. The lungs, kidneys, liver, and gross lesions from animals in the 70-and 200-ppm dosed groups were also examined. All tissues were preserved in 4% neutral formalin.

Results:

a. Organ Weights: No compound-related differences in organ weights or organ-to-body weight and organ-to-brain weight ratios were noted after 52 or 104 weeks of treatment. There were some significant decreases noted at the 12-month sacrifice but they did not occur in a dose-related manner nor at terminal sacrifice and were therefore not considered biologically significant.

b. Gross Pathology: The type and incidence of gross lesions observed in animals dying prior to or at terminal sacrifice were similar between control and treated groups. There was a high incidence of nodules or enlargement of the pituitary gland noted with the incidence being higher in females than males. This, however, was noted in both control and high-dose animals with comparable frequency.

c. Microscopic Pathology:

1) Nonneoplastic: The incidence of frequently observed nonneoplastic lesions is presented in Table 5. The type, incidence, and severity of nonneoplastic lesions was considered to be similar between control and treated groups.

2) Neoplastic: Table 6 represents the number and incidence of several neoplastic lesions observed after histopathological evaluation. The data for control and high-dose groups were extracted by the reviewer and expressed according to animal disposition. In mid- and high-dose groups only animals that died were routinely examined histologically. Slight increases in incidence of C-cell adenoma and follicular carcinoma of the thyroid gland were noted in high-dose (600-ppm) males but not females when compared to controls. There were also slight increases in incidence of malignant lymphoma in both male and female high-dose animals when compared to controls. A three-fold increase in incidence of mammary fibroadenomas was observed in the mid-dose (200 ppm) females but incidences were comparable between control and high-dose females. Although slight increases in incidence of several neoplastic lesions were evident, these increases were small and did not occur in a dose-related manner. Therefore, these increases were not considered to be

TABLE 5. Incidence of Frequently Observed Nonneoplastic Lesions in Rats Fed Thidiazuron Technical for 2 Years

Organ/Finding	Dietary Level (ppm)							
	Males				Females			
	0	70	200	600	0	70	200	600
Heart								
No. examined	60	9	13	60	60	16	17	60
Myofibrosis	15(25)[a]	3(33)	5(38)	24(40)	9(15)	4(25)	3(18)	17(28)
Nasal Cavity								
No. examined	60	9	13	60	60	15	17	60
Inflammation	15(25)	0 (0)	2(15)	28(47)	23(38)	7(47)	7(41)	37(62)
Kidneys								
No. examined	60	60	60	60	60	60	60	60
Lymphoid cell infiltration	5 (8)	5 (8)	13(22)	19(32)	15(25)	16(27)	15(25)	30(50)
Thyroid Gland								
No. examined	60	10	12	60	60	16	17	60
Ultimobranchial cyst	1 (2)	1(10)	0 (0)	5 (8)	4 (7)	2(13)	3(18)	2 (3)
C-cell hyperplasia	19(32)	3(30)	5(42)	25(42)	17(28)	0 (0)	5(29)	20(33)
Thymus								
No. examined	52	8	10	52	56	15	15	48
Lymphoid hyperplasia	4 (8)	3(38)	1(10)	10(19)	8(14)	9(60)	4(27)	14(29)

[a]No. of lesions (percent incidence).

TABLE 6. Incidence of Neoplastic Lesions in Rats Fed Thidiazuron Technical for 2 Years

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Organ/Finding	Dietary Level (ppm)							
	Males				Females			
	0	70	200	600	0	70	200	600
Thyroid Gland								
C-cell adenoma								
D/M[a]	0/11[b]	0/10	0/12	1/9	0/17	0/16	1/17	1/19
IS[a]	0/10	--	--	0/10	0/9	--	--	1/10
FS[a]	2/49	--	--	4/51	2/43	--	--	2/41
Total	2/70	0/10	0/12	5/70	2/69	0/16	1/17	4/70
Follicular carcinoma								
D/M	0/11	1/10	0/12	0/9	0/17	0/16	1/17	0/19
IS	0/10	--	--	0/10	0/9	--	--	0/10
FS	0/49	--	--	3/51	3/43	--	--	1/41
Total	0/70	1/10	0/12	3/70	3/69	0/16	1/17	1/70
Hemolymphoreticular System								
Malignant lymphoma								
D/M	1/11	1/9	4/13	3/9	2/17	0/15	5/17	4/19
IS	0/10	--	--	0/10	1/9	--	--	0/10
FS	3/49	--	--	4/51	5/43	--	--	4/41
Total	4/70	1/9	4/13	7/70	8/69	0/15	5/17	8/70
Mammary Glands								
Adenoma								
D/M	0/10	0/8	0/13	0/8	1/16	0/16	0/16	1/19
IS	0/10	--	--	0/10	0/9	--	--	0/10
FS	0/49	--	--	0/49	2/43	3/8	1/8	3/41
Total	0/69	0/8	0/13	0/67	3/68	3/24	1/24	4/70
Fibroadenoma								
D/M	0/10	0/8	0/13	0/8	1/16	2/16	5/16	4/19
IS	0/10	--	--	0/10	0/9	--	--	0/10
FS	0/49	--	--	0/49	6/43	2/8	4/8	2/41
Total	0/69	0/8	0/13	0/67	7/68	4/24	9/24	6/70
Adrenal Cortex								
Non-invasive tumor								
D/M	0/11	0/9	1/13	0/9	0/17	0/16	0/17	0/19
IS	0/10	--	--	0/10	0/9	--	--	0/10
FS	0/49	--	--	0/51	0/43	--	--	0/41
Total	0/70	0/9	1/13	0/70	0/69	0/16	0/17	0/70
Invasive tumor								
D/M	0/11	0/9	0/13	0/9	0/17	0/16	0/17	0/19
IS	0/10	--	--	0/10	0/9	--	--	0/10
FS	0/49	--	--	2/51	0/43	--	--	0/41
Total	0/70	0/9	0/13	3/70	0/69	0/16	0/17	0/70
Pituitary								
Adenoma								
D/M	8/11	4/9	6/12	5/9	14/17	12/15	15/16	14/19
IS	0/10	--	--	0/10	1/9	--	1/1	1/9
FS	15/49	3/11	8/8	22/51	36/43	23/23	21/21	28/41
Total	23/70	7/20	14/20	27/70	50/69	35/38	37/38	43/69

[a]Abbreviations used: D/M, dead or sacrificed moribund; FS, sacrificed at study termination; IS, interim sacrifice; (--), no data.

biologically significant. Incidences of other lesions observed were comparable between control and treated groups.

There were no increases in neoplastic lesions in animals that were sacrificed at 12 months.

D. STUDY AUTHOR'S CONCLUSIONS:

The study author concluded that there were slight dose-related decreases in body weight gain and food consumption for the 600-ppm male rats and a slight dose-related increase in mortality for the 600-ppm female rats after being fed thidiazuron technical for 2 years when compared to controls. There were no overt signs of toxicity or dose-related effects noted for clinical chemistry parameters or organ weights. There were no increases in incidence of gross or microscopic lesions at any site at any dietary level tested, i.e., 0, 70, 200, or 600 ppm. The study author assessed the NOEL for the study to be 200 ppm of thidiazuron technical.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The conduct and reporting of the study were satisfactory. The summary data were supported by individual data and recalculation of several mean and standard deviation values revealed no discrepancies. It should be noted, however, that summary data or individual animal data for ophthalmologic examinations or clinical observations were not provided. Histopathologic examination was satisfactory; loss of tissues due to autolysis or necropsy procedures was minimal. We agree with the author's assessment that there was no oncogenic response. Tumor incidences were comparable between control and treated groups and control incidences at specific sites were comparable to those usually found for this strain and age. However, it is apparent that the Maximum Tolerated Dose (MTD) was not achieved.

There were slight increases in incidences of myofibrosis, lymphoid cell infiltration in the kidneys, and lymphoid hyperplasia in the thymus of animals in the 600-ppm dose group when compared to controls (Table 5).

Slight, but sometimes significant differences in body weight were noted for 600-ppm males when compared to controls. Consistent significant decreases were observed during study weeks 73 through 89. Specified mean body weight gains as calculated by the reviewers are presented in Table 7. Statistical analysis (Anova and analysis of covariance) of several intervals reveals no significant differences. Total body weight gains for the 600-ppm males were approximately 20 g less than controls. In addition, food consumption values for the 600-ppm males were decreased (sporadic significance) when compared to controls. These reductions, although significantly different from controls, were usually only 1 to 2 g less and, therefore, were probably not of biological significance. No changes in body weights

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TABLE 7. Mean Body Weight Gains (±S.D.) of Rats Fed Thidiazuron Technical for 2 Years

Dietary Level (ppm)	Mean Body Weight Gain (g) at Study Week				
	13	51	77	89	104
			Males		
0	265±28	399±51	456±65	471±73	462±77
70	273±34	412±56	452±63	460±57	460±63
200	277±31	408±49	456±65	478±75	485±69
600	267±33	382±49	430±55	441±63	442±61
			Females		
0	131±17	198±34	225±49	239±53	251±61
70	135±15	198±32	239±35	259±40	269±47
200	135±16	201±33	240±43	253±46	266±51
600	134±16	198±31	237±47	252±53	265±58

or food consumption were noted for females at any dietary level. No biologically significant differences were noted for any clinical chemistry and hematology parameters, absolute and relative organ weights, or pharmacotoxic signs.

Although there were significant decreases in body weight and food consumption for 600-ppm males, these changes were slight and sporadic. Therefore, we assess that the dose levels for this study were not appropriately chosen to allow accurate interpretation of the oncogenic response. Under the conditions of the study, the LOEL for systemic toxicity was not achieved for females but was 600 ppm for males (31.7 mg/kg/day) and the NOEL for males was 200 ppm (10.6 mg/kg/day).

F. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 14-29.

APPENDIX A

Materials and Methods

Thidiazuron toxicology review

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