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DATA EVALUATION REPORT

Study Type: 92-Day Thyroid Function Study - Rats (Related to 83-1, 83-2, Study No. HLA 6123-112) 82-1

TOX Chem No: 454BB
PC No.: 108501
MRID No.: 420546-01

Test Material: AC 92,553, 92.6 percent pure

Synonyms: Pendimethalin, PROWL; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine

Study Number: T-0270

Sponsor: American Cyanamid Company
Princeton, NJ 08543

Testing Facility: Toxicology Department
American Cyanamid Company
Agricultural Research Division
Princeton, NJ 08543-0400

Title of Report: 92-Day Thyroid Function Study in Albino Rats with AC 92,553

Author: Joel E. Fischer

Report Issued: August 5, 1991

Conclusions: NOEL < 100 ppm (4.98 mg/kg/day)
LEL = 100 ppm (based on decreases in T_3 and T_4)

In addition, at 5000 ppm (245.4 mg/kg/day), there were decreases in body weight, body weight gain, increases in TSH levels, in absolute and relative thyroid weight and in the incidence and severity hypertrophy of thyroid follicular epithelial cells.

Classification: Supplementary based on design and intent.

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A. Materials

1. Test Compound - AC 92,553; Description: orange-yellow crystals; Lot No.: 5213-72A; Purity: 92.6 percent; Contaminants: not reported.

2. Test Animals - Species: rat; Strain: CD[Crl:CD(SD)]; Age: 13 weeks at start of feeding; Weight: males - 430 to 507 g, females - not tested; Source: Charles River Breeding Laboratories, Inc., Wilmington, MA.

B. Study Design:

1. Animal Assignment - Animals were randomly assigned to the following test groups.

92-Day Thyroid Function Study

Test Group	Dose in Diet (ppm)	Day of Sacrifice 15 No. Males	29 No. Males	57 No. Males	92 No. Males
Control	0	20	20	20	20
Low	100	20	20	20	20
High	5000	20	20	20	20

The rats were acclimatized to laboratory conditions for 5 weeks prior to start of feeding. The rats were individually housed in stainless-steel suspended cages with screen bottoms in a room at a temperature of 72 °F ± 4 °, relative humidity of 50 percent ± 20 percent and a 12-hour on/12-hour off light cycle. Food (Purina Certified Rodent Chow #5002 and water were provided ad libitum.

2. Diet Preparation - The diets were prepared by adding the proper amount of AC 92,553 to an initial premix of basal diet and blending in a Waring blender for 1 to 2 minutes. The premix was added to approximately 2 kg of basal diet and mixed for 2 to 3 minutes in a Hobart mixer. The amount was then transferred to a large Readco bowl mixer or Davis ribbon blender. The remainder of the basal diet was added and blended for 10 to 15 minutes. The test diets were prepared weekly and the test diet concentrations were adjusted to correct for the purity of the test material. At initiation of the study, batches of low- and high-concentration diets were analyzed for

homogeneity by measuring the top, middle, and bottom concentrations from left to right. Stability was determined by analyzing the diets from feed exposed for 8 days in the rat room.

Results - The concentration of the test substance (homogeneity sample) found in the 100 ppm test diet ranged from 89.7 to 109.0 percent with a mean of 97.5 percent and a coefficient of variation of 7.1 percent. The concentration of the test substance (homogeneity samples) found in the 5000 ppm diet ranged from 97.2 to 106.5 percent with a mean of 101.4 percent and coefficient of variation of 0.1 percent. The concentration of the test substance from the 100 ppm stability samples taken from feeders exposed for 8 days in the animal room averaged 92.4 percent. Stability samples taken from the 5000 ppm diet after 8 days averaged 106.1 percent. The average test diet concentration in the 100 ppm weekly diet ranged from 85.6 to 109.3 percent with a mean of 96.9 percent of the nominal concentration. The average test diet concentration in the 5000 ppm diet ranged from 94.5 to 110.5 percent with a mean of 101.4 percent of the nominal concentration.

3. Statistics - One-way analysis of variance (ANOVA) was used to analyze the following data: body weight, body weight gain, food consumption, TSH, T_3, T_4, thyroid gland weight, and thyroid gland-body weight ratios. If ANOVA was significant, then a Dunnett's t-test was used for pairwise comparisons between the treated and control groups. The level of significance was at the 5.0 percent probability level. It should be noted that data for TSH are also presented as adjusted data by removing outlying data points (points $\pm$ 1.5 SD) to reduce data variability. Excluded points are identified in the individual animal data tables.

C. Methods and Results:

1. Observations - All animals were inspected for mortality and signs of toxicity. Once a week each rat was removed from its cage and carefully examined for abnormalities and clinical signs of toxicity.

Results - Two rats, one control (#17) and one rat (#151) in the 100 ppm group died on Days 15 and 39, respectively. No cause of death was evident. No other deaths occurred. Most of the treated rats had yellow-to-amber colored urine beginning on Day 2 in the 5000 ppm group and on Day 8 in the 100 ppm animals. Additionally, several animals in the 5000 ppm group had yellow stained

coats. (This has been observed in previous studies and is believed to be a result of contact with metabolites of the test substance or the test substance itself.)

2. Body Weight - Individual animal body weights were determined initially (Day 0), and weekly thereafter.

Results - Body weight was significantly decreased in the 5000 ppm at the majority of the measurement periods when compared to the controls. The decrease ranged from 4.4 percent at Week 3 to 6.5 percent at Week 6. Body weights of the control and 5000 ppm group were not significantly different after Week 10: Body weight gain was significantly decreased (≈20%) in males in the 5000 ppm group when compared to controls over the length of the study. Body weight gain of rats in the 100 ppm group were decreased by approximately 8.7 percent when compared to controls over the length of the study (see Table 1).

Table 1: Body Weight and Percent (%) Decrease and Body Weight Gain Compared to Controls

Dose Level (ppm)	0	3	6	9	13	Initial-13
0	467.5	531.9	578.1	619.2	665.4	193.8
100	469.5	526.0	575.8	594.2	644.5	177.0
		(1.0)	(0.4)	(4.0)	(3.0)	(-8.7)
5000	471.5	508.7*	540.7*	582.6*	633.2	154.4*
		(4.4)	(6.5)	(5.9)	(4.8)	(-20)

* $p < 0.05$

3. Food Consumption and Compound Intake - Individual food consumption data were collected weekly.

Results - Food consumption was statistically decreased by approximately 30, 2.7, 6.9, and 8.2 percent for males in the 5000 ppm group at Weeks 1, 2, 4, and 7. In general, food consumption was only slightly decreased in males in the 5000 ppm group at most measurement intervals when compared to the controls. Food consumption was decreased by approximately 5 percent in the males in the 100 ppm group when compared to controls at Week 1, but was otherwise comparable to the controls. The time weighted average compound intake was 4.98 and 245.4 mg/kg/day of the test substance in the 100 and 5000 ppm groups of animals, respectively.

4. Blood samples were collected from 20 rats per dose level on Day 15, 29, 57, and 92 of the study by cardiac puncture and allowed to clot for 1 hour at room temperature, centrifuged and the serum separated into 4 aliquots of approximately 1 mL each and the samples frozen and stored at -80 °C. Animals were not fasted overnight. The samples were shipped to Hazleton Laboratories, Inc. in Vienna, VA for analysis of thyroid stimulating hormone (TSH), triiodothyronine (T_3) and thyroxine (T_4).

   Results - There were statistically significant increases in TSH in males in the 5000 ppm group on Day 29 (91%) and Day 57 (71%) and an increase (not significant) on Day 92 of approximately 66 percent. T_3 levels were significantly decreased by approximately 18 to 40 percent at all measurement intervals during the study in males in the 5000 ppm group. T_3 levels in males in the 100 ppm group were significantly decreased by 18 and 23 percent on Days 15 and 29, respectively. Levels of T_3 in males in the 100 ppm group was comparable to controls on Days 57 and 92. T_4 levels were significantly decreased by approximately 67 to 73 percent at all measurement intervals during the study in males in the 5000 ppm group when compared to controls. Levels of T_4 were significantly decreased by approximately 18 to 28 percent in males in the 100 ppm group on Days 57 and 92 when compared to controls. T_4 levels of males in the 100 ppm group were comparable to controls on Days 15 and 29 (see Table 2). Figures 1, 3 and 4 from the study report are attached to this DER.

5. Sacrifice and Pathology - Twenty rats per group were sacrificed at Days 15, 29, 57, and 92. Thyroid glands were removed intact, attached to the trachea. Thyroid and adjacent tissues were fixed in 10 percent neutral phosphate-buffered formalin. After at least 24 hrs of fixation, the thyroid lobes were carefully dissected from the trachea and connective tissue, blotted dry on filter paper and the combined weight of the left and right lobes recorded. All samples of thyroid gland were submitted to W.R. Brown, D.V.M., Ph.D., veterinary pathologist, New Britain, PA for processing and histological evaluation. The tissues were cut, blocked in paraffin, sectioned to 4 to 6 μ, mounted on slides, stained with H&E and examined microscopically.

TABLE 2 Levels of Serum TSH, T_3 and T_4 in Male Rats (% increase or decrease)

Weeks / Dose (ppm)	15	29	57	92
		TSH (ng/ml)		
0	4.35	4.02	3.87	4.90
100	3.99 (-.08)	4.69 (16.7)	4.67 (20.7)	4.20 (-1.4)
5000	5.12 (17.8)	7.66* (90.5)	6.62* (71.0)	6.65 (35.7)
		T_3 (ng/ml)		
0	69.69	86.96	71.07	65.71
100	57.42* (-17.6)	67.17* (-19.8)	71.08 (0.0)	66.11 (0.6)
5000	41.91 (-39.9)	63.94* (-26.5)	58.43* -17.8)	54.14* (-17.6)
		T_4 (ug/ml)		
0	4.26	4.01	4.03	4.62
100	3.74 (-12.2)	3.82 (-4.7)	3.30* (-18.1)	3.32* (-28.1)
5000	1.37* (-67.8)	1.34* (-66.6)	1.08* (73.2)	1.33* (-70.8)

* $p < 0.05$

Results

a. Organ Weight - See attached Table 5.7.1 taken from the study report. There was a dose-related increase in the absolute and relative thyroid weights of males in the 100 and 5000 ppm groups when compared to controls at all measurement intervals. The increase in absolute thyroid weight ranged from 6.9 to 14.5 percent of control values for animals in the 100 ppm group and from 23.8 to 35.5 percent of control values for animals in the 5000 ppm group. Relative thyroid to body weight decreased from 0 to 16.7 percent of control values for animals in the 100 ppm group and from 30.8 to 50.0 percent of control values for animals in the 5000 ppm groups. Statistically significant increases were observed in only the 5000 ppm group, but at all measurement intervals when compared to controls.

b. Histopathology - There was an increase in the incidence and severity (slight-to-moderate) of hypertrophy of thyroid follicular epithelial cells in males in the 5000 ppm on Day 57 (70%) and Day 92

(80%) when compared to controls on Day 57 (20%) and Day 92 (20%). (See Table 3.)

Table 3. Incidence of Hypertrophy of the Thyroid Follicular Epithelial Cells in Males

	Day 15			Day 29		
Dose level (ppm)	0	100	5000	0	100	5000
No. Animals	20	19	20	20	20	20
Hypertrophy						
- minimal	5	3	6	3	3	4
- slight	0	0	1	0	0	2
- moderate	0	0	0	0	0	0
- total incidence	5/20 (25)[1]	3/19 (16)	7/20 (35)	3/20 (15)	3/20 (15)	3/20 (15)
	Day 57			Day 92		
No. Animals	20	19	20	20	20	20
Hypertrophy						
- minimal	3	2	4	3	4	6
- slight	1	2	8	1	1	6
- moderate	0	0	2	0	0	4
- total incidence	4/20 (20)	4/19 (22)	14/20 (71)	4/20 (20)	5/20 (25)	16/20 (80)

[1]percent incidence

D. Discussion

The preparation of the diet was adequate with respect to weekly concentrations, homogeneity of the diets and stability of the diets stored over an 8-day period at room temperature. Most of the rats in the 2 treatment groups had yellow to amber colored urine that was attributed to staining with the metabolites of the test substance or with the test substance itself. Rats in the 5000 ppm group also had yellow stained coats. Mortality was not affected by administration of the test material and no signs of clinical toxicity were apparent. Body weight was significantly

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decreased in rats in the 5000 ppm group over most of the measurement intervals. Body weight gain was significantly decreased (≈ 20%) in rats in the 5000 ppm group when compared to controls. Body weight gain was only slightly decreased (≈9%) in the 100 ppm group when compared to controls. Food consumption was significantly decreased in rats in the 5000 ppm group at most measurement intervals when compared to controls. The decreases were considered to be only marginal. The 30 percent decrease at Week 1 was significant but can probably be related to a decrease in palatability. Serum TSH levels were significantly increased in the 5000 ppm group when compared to controls. Serum T_3 and T_4 levels were significantly decreased in rats in the 5000 ppm groups at all measurement intervals. Serum T_3 levels were also significantly decreased in rats in the 100 ppm group on Days 15 and 29. Serum T_4 levels were significantly decreased in the 100 ppm group on Days 57 and 92. Thyroid absolute and relative weights were decreased in a dose-response relationship at many measurement intervals. Statistical significance was only achieved at the 5000 ppm level. Thyroids of rats in the 5000 ppm group exhibited an increase in the incidence and severity of hypertrophy of follicular epithelial cells at Days 57 and 92.

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