

US EPA ARCHIVE DOCUMENT

13000

4

Reviewed by: William B. Greear, M.P.H. *William B. Greear 11/21/94*
 Review Section IV, Toxicology Branch I (7509C)
 Secondary Reviewer: Marion P. Copley, D.V.M. *Marion Copley 11/24/94*
 Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: 56-Day Thyroid Function Study (82-SS)

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

Tox Chem No. 454BB
 MRID No. 431350-01
 PC No. 108501
 DP Barcode No. D201875
 Submission No. S463228

TEST MATERIAL: AC 92,553, 92.6%

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

STUDY NUMBER: AX93-1, L-2366

SPONSOR: American Cyanamid Company
 Princeton, NJ 08543

TESTING FACILITY: Toxicology Department
 American Cyanamid Company
 Princeton, NJ 08543

TITLE OF REPORT: 56-Day Thyroid Function Study in Albino Rats with AC 92,553

AUTHOR(S): Joel E. Fischer

REPORT ISSUED: May 28, 1993

EXECUTIVE SUMMARY: In a metabolism study, groups of 65 to 70 male Crl:(SD) rats were administered 500 or 5000 ppm (31 and 292 mg/kg/day) of pendimethalin (92.6%) in the diet for 28 days. A recovery period of up to 28 days was employed.

There were no deaths or clinical signs of toxicity during or after the treatment period at either dose. At 500 ppm there were decreased total T_4 (38%), rT_3 (25%) and total free T_4 (28%); increased percent free T_3 (13%), ~~percent free T_3 (13%) and total free T_4 (28%)~~, increased follicular cell height (40%) and decreased area occupied by colloid (51%) during treatment. At 5000 ppm, body weight (8%), body weight gain (29%) and food consumption (15%) were decreased compared to controls during the treatment period. Thyroid changes during treatment with 5000 ppm included: increased absolute (15%) and relative thyroid weight (23%); decreased total T_4 (74%), total T_3 (25%), rT_3 (36%), total free T_4 (40%), and [^{125}I]- T_4 to transthyretrin binding; increased

percent free T_4 (117%), percent free T_3 (26%) and [^{125}I]- T_4 to albumin binding; increased follicular cell height (75%) and decreased area occupied by colloid (45%); ultrastructural thyroid changes were consistent with mild to moderate TSH stimulation except for the accumulation of dense-bodies in the cytoplasm which may be reaction products of AC 92,553. Most parameters were reversible after treatment subsided except for a slight decreased body weight compared to controls (7%) at 5000 ppm. There were no changes in TSH, total free T_3 or diameter of follicle cells.

This special mechanistic study is **Core-Supplementary**. It was not designed to satisfy any guideline requirement.

A. Materials

1. **Test compound:** AC 92,553;
Description: not reported
Lot#: AC 52113-72A
Purity: 92.6%
2. **Test animals:**
Species: rat
Strain: Crl:CD(SD)
Age: 11 weeks
Sex: males
Body weight: 347-408g
Source: Charles River Breeding Laboratories, Inc.
Wilmington, MA

B. Study Design

1. Animal assignment - Rats were randomly assigned to the following test groups (see Table 1).

<u>Table 1. Animal Assignment</u> <u>56-Day Thyroid Function Study</u>								
Test Group	Dose in Diet (ppm)	Day of Sacrifice						
		-3	3	7	10	14	28	56
Control	0	15	5	5	5	5	5	70
Low	500	0	5	5	5	5	5	65
High	5000	0	5	5	5	5	5	70

The animals received the test diets for 28 days followed, or end of treatment, by a 28 day observation period. The rats were acclimated to laboratory conditions for 3 weeks prior to the start of feeding. The rats were individually housed in stainless steel, suspended, screen-bottomed cages in a room with temperature of $72 \pm 4^\circ\text{F}$, a relative humidity of $50 \pm 20\%$ and with a 12-hour light/dark period. Food (Purina Certified Rodent Chow #5002) and water were provided ad libitum.

2. Diet preparation: The diets were prepared weekly by adding the appropriate amount of AC 92,553 to an initial premix of basal diet and blending in a Waring blender for 1 minute. Corrections were made based on purity of the test material. The premix was added to approximately 2 kg of basal diet, mixed in a Hobart mixer for 2 minutes and then transferred to a large barrel mixer. The remaining basal diet was then added and blended for 25 minutes. At initiation and at week 1, 2, 3 and 4, batches of the diets from the 500 and 5000 ppm groups were used to determine the homogeneity of the test diets. The stability of AC 92,553 was not determined because the stability of AC 92,553 in the diet had already been determined in prior studies (see MRID# 420546-01; T-270; 8/5/91).

Results: The diets showed good homogeneity. The test diets contained 89-96% of their expected values.

3. Statistics: One way analysis of variance (ANOVA) was used to analyze body weights, body weight gains, food consumption, thyroid gland weights and thyroid gland-body weight percentages. If significant, a Dunnett's t-test was used for pairwise comparisons between groups.

C. Methods and Results

1. Observations: Animals were observed daily for death and clinical signs of toxicity. Once a week the animals received a more detailed examination.

Results: No deaths occurred. The urine of rats in the test group was yellow throughout the study.

2. Body Weight: Determined at initiation and weekly thereafter.

Results: Body weights were significantly decreased for rats in the 5000 ppm group both during the treatment and recovery phases. At day 29, males in the 5000 ppm

group had body weights that were 7.6% lower than the controls. At day 56, rats in the 5000 ppm group had body weights that were 6.7% lower than the controls. Body weight gain of rats in the 5000 ppm group was also significantly (29.4%) less than the controls from day 0-29. No difference in body weight gain was observed between the control and 5000 ppm groups from day 29 to 56 (see Table 2).

Dose Level (ppm)	Day						
	0	15	29	43	56	0-29	29-56
0	392.1	440.9	481.1	509.8	543.7	96.9	62.0
500	390.3	441.6	483.5	506.4	537.7	93.7	53.6
5000	388.2	414.7*	444.6*	477.9*	507.1*	68.4*	59.1

¹ Data abstracted from Table 5.3.1, p. 32
² Data abstracted from Table 5.3.2, p. 34
* Significantly different from controls at p < 0.05

3. Food Consumption and Compound Intake: Collected weekly

Results: Rats in the 5000 ppm group had significantly reduced food consumption during the 28 day treatment period (17%) and slightly reduced food consumption during the 28 day recovery period (8.7%). Rats in the 500 ppm group also exhibited slightly reduced food consumption during the treatment period (8.8%) but not during the recovery period (see Table 3).

Dose Level (ppm)	Days				
	0-8	8-15	22-29	35-43	50-56
0	213.4	191.7	197.2	203.3	173.8
500	204.4	184.6	179.9*	202.7	178.0
5000	175.7*	174.0*	162.5*	193.8	158.6

¹ Data extracted from Table 5.2.1, p. 30
* significantly different at p < 0.05

Average compound intake was 31 and 292 mg/kg/day, respectively, for rats in the 500 and 5000 ppm groups during the treatment period.

4. Thyroid Gland and Pituitary Weights: Determined at sacrifice days 3, 7, 10, 14, 28 and 56.

Absolute and relative thyroid weights and were significantly increased in rats in the 5000 ppm group on day 28 by 15% and 23%, respectively. Absolute and relative thyroid weights were somewhat increased by 3% and 5%, respectively, in the 500 ppm group when compared to controls on day 28, but the increases were not statistically significant. At the end of the recovery period absolute and relative thyroid weights were comparable between the control, 500 and 5000 ppm groups. Pituitary weight did not appear to be affected by treatment.

Organ	Control	500 ppm	5000 ppm
<u>Day 28</u>			
Thyroid (mg)	20.680	21.300	23.733*
% Bwt.	0.0043	0.0045	0.0053*
<u>Day 56</u>			
Thyroid (mg)	19.033	21.653	20.453
% Bwt.	0.0035	0.0041	0.0041

* statistically significant at $p < 0.05$

Data extracted from Table 5.4.1, p. 37 of MRID# 43135001

5. Thyroid Hormones: At each sacrifice, serum levels of T_3 , rT_3 , T_4 and TSH were determined. Free T_3 and T_4 in the serum were also analyzed on day 3, 28 (end of treatment) and 56 (end of recovery period). The binding at $[^{125}I]-T_4$ to albumin and transthyretin (a serum protein) was measured on day 28.

Results:

TSH: TSH was not affected by treatment (data not presented).

Total Serum T_4 : T_4 was decreased in the 5000 ppm group at all time points during the treatment period, sometimes up to 74%, but not on day 56. T_4 was also decreased in the 500 ppm group at all time points

during treatment, sometimes up to 38%, but not on day 56 (see Table 4).

Total Serum T_3 : T_3 was decreased in the 5000 ppm group, sometimes up to 25% during the treatment period, but not on day 56 (see Table 4). T_3 levels were not affected in the 500 ppm group.

Serum rT_3 : rT_3 was decreased in the 5000 ppm group, sometimes up to 36% on days 3, 7, 10 and 28, but not at day 56 (see Table 4). rT_3 was decreased in the 500 ppm group on day 28 by 25%, but not on day 56 (see Table 4).

Percent Serum Free T_4 : Percent free T_4 levels were increased in the 5000 ppm group by up to 117% on days 3 and 28, but not on day 56. Percent free T_4 levels were also increased in the 500 ppm group by 33% on day 3, but not on day 56 (see Table 4).

Percent Serum Free T_3 : Percent free T_3 was increased in both the 500 and 5000 ppm groups by up to 13% and 26%, respectively, on days 3 and 28, but not on day 56 (see Table 4).

Serum Total Free T_4 : Total free T_4 was decreased in the 5000 ppm group only on days 3 and 28 by up to 40%, and in the 500 ppm group on day 28 by 28%, but not at day 56 (see Table 4).

Serum Total Free T_3 : Total free T_3 was comparable among the control and treated groups at all time points (data not presented).

Analysis of Serum Binding of T_4 to Transport Protein: Rats in the 5000 ppm group exhibited an increase in the percent binding of [125 I]- T_4 to albumin and a decrease in the percent binding of [125 I]- T_4 to transthyretin (see Figure 1-taken from p.67 of the study report).

- Morphometric Analysis: Evaluation of the diameter of thyroid follicles, area of the follicle occupied by colloid and height of the follicular cells were determined on days 3, 7, 10, 14, 28 (end of treatment) and 56 (end of recovery). Electron microscopy (EM) was also used on an additional groups of rats on day 28.

Results: Follicular cell height was increased in rats in the 500 ppm group by up to 40% and 5000 ppm groups by up to 75% on days 3, 7, 10, 14 and 28. Follicular cell height was only increased in the 500 ppm group by 19% on day 56. The increase in the 500 ppm group on

day 56 is nominal and may represent normal biological variation considering the reversibility of the effect in the 5000 ppm group (see Table 4). The follicle area occupied by colloid was decreased in the 500 ppm group on days 3, 7, 10, 14 and 28 by up to 51%, but not on day 56. The area occupied by colloid was also reduced in the 5000 ppm group on 7, 10, 14 and 28 days by up to 50%, but not on day 56 (see Table 4). The diameter of thyroid follicles in the 500 ppm group decreased on days 7 and 14 by up to 14%, and increased in the 5000 ppm group by 10% on day 28. The diameter of the thyroid follicles were comparable among groups on day 56 (see Table 4). The changes in thyroid follicle diameter were small and, perhaps, represent normal biological variation.

EM Evaluation: There was a greater accumulation of dense bodies in follicular cells in the 5000 ppm group. The bodies were irregular in shape, often membrane-limited, and filled the cytoplasmic area and crowded other synthetic and secretory organelles. The thyroid follicular cells also had extensive, often dilated endoplasmic reticulum, prominent Golgi apparatuses with small granules, numerous colloid droplets, and large mitochondria. The luminal surface of follicular cells had closely situated, long microvilli and occasional cytoplasmic projections into the colloid. The pathologist indicated that the ultrastructural changes in the follicular cells were consistent with a mild to moderate TSH stimulation, except for the accumulation of the dense bodies in the cytoplasm which may be a reaction product of AC 92,553 with thyroid peroxidase or lysosomal enzymes in the follicular cells.

D. DISCUSSION

There were no deaths or clinical signs of toxicity during or after the treatment period at either dose. At 500 ppm there were decreased total T_4 (38%), rT_3 (25%) and total free T_4 (28%); increased percent free T_3 (13%), percent free T_3 (13%) and total free T_4 (28%), increased follicular cell height (40%) and decreased area occupied by colloid (51%) during treatment. At 5000 ppm, body weight (8%), body weight gain (29%) and food consumption (15%) were decreased compared to controls during the treatment period. Thyroid changes during treatment with 5000 ppm included: increased absolute (15%) and relative thyroid weight (23%); decreased total T_4 (74%), total T_3 (25%), rT_3 (36%), total free T_4 (40%), and [^{125}I]- T_4 to transthyretin binding; increased percent free T_4 (117%), percent free T_3 (26%) and [^{125}I]- T_4 to albumin binding; increased follicular cell height (75%) and

decreased area occupied by colloid (45%); ultrastructural thyroid changes were consistent with mild to moderate TSH stimulation except for the accumulation of dense-bodies in the cytoplasm which may be reaction products of AC 92,553. Most parameters were reversible after treatment subsided except for a slight decreased body weight compared to controls (7%) at 5000 ppm. There were no changes in TSH, total free T_3 or the diameter of follicle cells.

This special mechanistic study is **Core-Supplementary**. It was not designed to satisfy any guideline requirement.

Table 4. Select Thyroid Parameters in Serum

Parameter	Day 28		
	Control	500 ppm	5000 ppm
Total T ₄ (μg/dl)	5.85±.26	3.65±0.28*	2.25±.33**
Total T ₃ (ng/dl)	73.5±3.2	65.6±3.9	54.9±4.4*
rT ₃ (pg/ml)	97.5±6.7	73.6±6.8*	77.2±7.7*
% Free T ₄	.030±.000	.032±.003	.051±.005*
% Free T ₃	0.36±0.01	0.41±0.01*	0.40±0.01*
Total Free T ₄ (ng/dl)	1.55±0.06	1.11±0.10*	1.01±0.08*
Thy. Cell Hgt. (μ)	7.42±.16	10.42±.24**	12.97±.25**
Follicle Diam. (μm)	44.2±.84	45.8±.64	48.4±.83*
Colloid Area (0 ² x10 ²)	6.14±.41	4.47±.23*	3.39±.33*

	Day 56		
Total T ₄ (μ/dl)	5.68±.30	6.27±.25	6.26±.21
Total T ₃ (ng/dl)	85.0±5.0	80.1±3.8	77.4±3.9
rT ₃ (pg/ml)	96.9±5.3	94.2±4.9	97.2±7.5
% Free T ₄	.030±.000	.029±.001	.029±.002
% Free T ₃	0.34±0.01	0.35±0.01	0.32±0.02
Total Free T ₄ (ng/dl)	1.88±0.21	1.88±0.11	1.96±0.10
Thy. Cell Hgt. (μ)	8.38±.19	9.98±.17***	8.5±.22
Follicle Diam. (μm)	43.0±1.57	43.5±.81	43.5±1.55
Colloid Area (μ ² x10 ²)	7.11±.86	4.97±.38	5.99±.55

* significantly different from controls (p < 0.05)

** significantly different from control and each other (p < 0.05)

+ significantly different from 500 ppm group (p < 0.05)

*** significantly different from control and 5000 ppm group (p < 0.05)

Data extracted from tables 2,3,4,5,6,7,8,I,II and III; pp. 13,15,17,19,21,23,25 106,107 and 108, respectively, of MRID# 431350-01.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TOX
ONELINERS

P. C. No: 108501	TOXCHEM NO.: 454BB	Chemical Name: Pendimethalin	CORE GRADE DOC.#
CITATION (82-SS) Special Study Species: rat Lab. Name: An. Cytomid Study No: AX93-1, I-2366 Date: 5/28/93	MATERIAL NUMBER AC 92,553 Lot AC 52113-72A	MRID NUMBER 431350-01	TOX CAT
RESULTS In a metabolism study, groups of 65 to 70 male Cri:(SD) rats were administered 500 or 5000 ppm (31 and 292 mg/kg/day) of pendimethalin (92.6%) in the diet for 28 days. A recovery period of up to 28 days was employed. There were no deaths or clinical signs of toxicity during or after the treatment period at either dose. At 500 ppm there were decreased total T ₄ (36%), FT ₄ (25%) and total free T ₄ (26%); increased percent free T ₄ (13%), percent free T ₃ (13%) and total free T ₃ (26%), increased follicular cell height (40%) and decreased area occupied by colloid (51%) during treatment. At 5000 ppm, body weight (8%), body weight gain (29%) and food consumption (15%) were decreased compared to controls during the treatment period. Thyroid changes during treatment with 5000 ppm included: increased absolute (15%) and relative thyroid weight (23%); decreased total T ₄ (74%), total T ₃ (25%), FT ₄ (36%), total free T ₄ (40%), and [¹²⁵ I]-T ₄ to transthyretin binding; increased percent free T ₄ (117%), percent free T ₃ (26%) and [¹²⁵ I]-T ₃ to albumin binding; increased follicular cell height (75%) and decreased area occupied by colloid (45%); ultrastructural thyroid changes were consistent with mild to moderate TSH stimulation except for the accumulation of dense-bodies in the cytoplasm which may be reaction products of AC 92,553. Most parameters were reversible after treatment subsided except for a slight decreased body weight compared to controls (7%) at 5000 ppm. There were no changes in TSH, total free T ₄ , or diameter of follicle cells.			Suppl ement ary

13544

R059060

Chemical:	Pendimethalin
PC Code:	108501
HED File Code	13000 Tox Reviews
Memo Date:	11/29/94 12:00:00 AM
File ID:	DPD201875
Accession Number:	412-04-0140

HED Records Reference Center
04/02/2004