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DATA EVALUATION REPORT

STUDY TYPE: 2-year onco - rat(male)(83-5)

TOX. CHEM NO: 454BB
PC No.: 108501
MRID NO.: 420278-02

TEST MATERIAL: Pendimethalin

SYNONYMS: AC 92,553

STUDY NUMBER: HLA 362-191

SPONSOR: American Cyanamid

TESTING FACILITY: Hazleton Laboratories America, Inc. Rockville, Maryland

TITLE OF REPORT: Effects of chronic dietary administration of AC 92,553 on the function and structure of male rat thyroids.

AUTHOR(S): D.E. Bailey

REPORT ISSUED: 9/10/91

CONCLUSION: NOEL systemic < 1250 ppm (51 mg/kg/day)
LEL < 1250 ppm based on non-neoplastic thyroid follicular cell changes and increased liver weight. In addition there was an increase in thyroid weight and non-neoplastic liver pathology at 2500 ppm and above. There was a decrease in body weight gain at 3750 ppm. At 5000 ppm (213 mg/kg/day) GGT and total cholesterol were increased.
Thyroid function NOEL = 3750 ppm (154 mg/kg/day)(not definitive).
LEL = 5000 ppm based on a TSH increased.
Thyroid pathology NOEL < 1250 ppm.
LEL ≤ 1250 ppm based on decreased colloid and increase of cysts. At 2500 ppm (103 mg/kg/day) there was an increase of pigment and hypertrophy of follicular cells. At 3750 ppm and above there was also hyperplasia. Thyroid follicular adenomas were increased at 5000 ppm.

Male Sprague Dawley (Crl:CD(SD)BR) rats were exposed to 0, 1250, 2500, 3750 or 5000 ppm (approximately 0, 51, 103, 154 or 213 mg/kg/day) in the diet for up to 2 years.

Classification: core-supplementary. Does not satisfy the guideline requirements for a chronic oncogenicity rodent study due to the study design and purpose of this study.

Special Review Criteria: (PR 54) There is an increase in male thyroid tumors.

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A. MATERIALS:

1. Test compound: Pendimethalin (AC 92,533). Description - yellowish-orange solid, Lot # - AC 5213-72A, Purity - 92.6%, stored in ambient conditions.

2. Test animals: Male, Species: Rat, Strain: Sprague Dawley (Crl:CD(SD)BR), Age: 36 days old, Weight (range): 121.9-169.5 gm, Source: Charles River Labs, Portage, Michigan. Acclimatization: 1 week. Animals were individually housed in environmentally controlled rooms.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned by a computer generated weight randomization program to the test groups as noted in table 1. Animals were exposed to the test compound in the diet daily for 2 years.

TABLE 1 Experimental design, males only

Test Group	ppm in diet	weeks on diet for interim and final sacrifices 1	13	26	39	52	104
1 Cont.	0	15	15	15	15	15	50
2 Low (LDT)	1250	15	15	15	15	15	50
3 Mid 1(MDT1)	2500	15	15	15	15	15	50
4 Mid 2(MDT2)	3750	15	15	15	15	15	50
5 High (HDT)	5000	15	15	15	15	15	50

2. Compound/diet preparation

Pendimethalin/diets were prepared weekly. Adjustment was made for purity. Compound was crushed in a mortar and pestle then premixed in a blender with about 200 grams of feed. This was added to additional feed in a mixer resulting in the final test diet. Prior to the study samples of the 1250 and 5000 ppm test diets were taken at preparation, and 7 and 14 days and submitted to the sponsor for analysis. All levels were sampled weekly.

Results - Stability, homogeneity and concentration appeared to be adequate based on data presented in the study.

3. Animals received food (Purina lab rodent chow #5002) and water ad libitum.

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4. Statistics - The procedures utilized in analyzing the numerical data are in attachment 1 taken from the study report.

5. Signed GLP and quality assurance statements are present.

C. METHODS AND RESULTS:

1. Observations - Animals were inspected at least twice daily for signs of toxicity and mortality. Detailed observations were made weekly.

   Toxicity/Mortality (survival) - Survival did not appear to be affected by treatment with 52, 36, 40, 36 and 40% survival for controls to the high dose.
   Signs of toxicity were sporadic, not dose related and were not considered treatment related. Urine in treatment groups was slightly orange to darker orange/brown. This was probably due to the test material (metabolites).

2. Body weight - Animals were weighed weekly.

   Body weight gain for the 104 weeks of the study are decreased 8, 14, 21 % for the 2500, 3750 and 5000 ppm groups, respectively, when compared to controls. As can be seen in table 3 (table 3B taken from the study report) weight gain is statistically decreased in 2500 ppm and above. However, this statistical decrease is only greater than 10% of controls at 5000 ppm (15 - 36 % throughout most of the study), and at 2500 ppm (11 %) and 3750 ppm (25%) after week 39 for the rest of the first year.

TABLE 3 (taken from the study report)

TABLE 3B
MEAN BODY WEIGHT CHANGE (G)
CHRONIC DIETARY OF AC 92,553

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GROUP AND DOSE LEVEL (PPM)		WEEK 0 - 1	0 - 13	0 - 26	0 - 39	0 - 52	0 - 104
				MALES			
1	MEAN	60.3	406 5	512 3	568.9	625 5	558 5
	S.D.	5.17	42.63	59 46	75.39	81 51	104 10
.000	N	125	110	95	80	65	26
2	MEAN	61.1	397.2	505 9	565 2	614.9	569 4
	S.D.	5.58	45 27	57 95	69 14	81 90	130 41
1250.000	N	125	109	94	79	63	18
3	MEAN	56.4*	386.1*	488.2*	541.8	592 1*	511 3
	S.D.	6.11	39 01	49.59	57.96	71 53	128 11
2500.000	N	125	110	94	79	64	21
4	MEAN	49 4*	374 0*	472 0*	526 7*	569 1*	481 0
	S.D.	8 16	34 64	49 10	58 72	73 52	115 63
3750.000	N	125	110	94	79	64	19
5	MEAN	44 3*	348 7*	446 2*	492 4*	528 4*	439 4*
	S.D.	7 66	33 36	46 27	49 18	57 80	129 34
5000 000	N	125	110	95	79	64	20

* Significantly different from control value, $p \leq 0.05$.

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3. Food consumption and compound intake - Food consumption was measured weekly.

Food consumption (g/animal) (see table 4 taken from the study) was decreased statistically in the 5000 ppm group from week 1 - 26, in the 2500 and 3750 ppm groups from week 1 - 13. None of these decreases were greater than 8 %and were therefore probably not related to treatment.

TABLE 4 (taken from the study report)

TABLE 4B HLA 362191
MEAN TOTAL FOOD CONSUMPTION AND STANDARD DEVIATIONS (G)
CHRONIC DIETARY OF AC 92,553

GROUP AND DOSE LEVEL (PPM)		WEEK 1	1 - 13	1 - 26	1 - 39	1 - 52	1 - 104
				MALES			
1	MEAN	161.3	2388.7	4693.4	6968.4	9312.7	18598.6
	S.D.	11.52	126.91	272.85	403.00	551.66	837.99
.000	N	124	70	55	39	32	7
2	MEAN	162.3	2372.6	4687.9	7035.2	9382.2	18731.5
	S.D.	9.83	131.50	251.63	351.3[illegible]	518.10	706.96
1250.000	N	123	70	59	45	29	2
3	MEAN	157.8	2316.6 *	4624.1	6986.0	9373.8	18436.0
	S.D.	10.72	139.76	294.13	399.83	539.41	1453.53
2500.000	N	99	74	58	42	31	3
4	MEAN	151.9 *	2266.2 *	4582.7	6948.3	9234.3	19134.6
	S.D.	18.69	144.71	299.16	426.73	621.13	486.87
3750.000	N	68	49	38	28	21	3
5	MEAN	146.8 *	2220.6 *	4510.8 *	6806.5	9073.8	18009.2
	S.D.	21.69	122.55	273.49	413.35	471.35	1708.51
5000.000	N	65	48	32	24	18	2

* Significantly different from control value, $p \leq 0.05$.

Compound intake - based on body weight and food consumption was presented in ranges with the highest consumed dose during the first 2 weeks of the study, decreasing for the next 14 weeks and plateauing for the remainder of the study. The time weighted average (calculated by the EPA reviewer)are presented in table 5.

TABLE 5 Compound Intake - Time Weighted Average (mg/kg/day)

Concentration in Diet (ppm)	1250	2500	3750	5000
Dose week 1-13	85	168	211	338
week 1-104	51	103	154	213

4. Ophthalmological examination - This was not mentioned

5. Blood was collected from the 15 rats (fasted) scheduled for interim (weeks 1, 14, 27, 40, 53 and 104) and terminal sacrifices for clinical analysis (hematology was not performed). The CHECKED (X) parameters were examined.

   a. Hematology not performed

   b. Clinical Chemistry - general

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulin and A/G ratio
X	Sodium*	X	Glucose*
	Enzymes	X	Total (and direct) bilirubin
X	Alkaline phosphatase (ALK)	X	Total serum Protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatinine phosphokinase*		Serum protein electrophoreses
X	Lactic acid dehydrogenase (LAD)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for subchronic and chronic studies

Results - There was an increase in serum total cholesterol and GGT at most time points in the 5000 ppm group (see table 6). Statistical changes at other doses and in other parameters were probably not treatment-related since they only occurred at sporadic time points, were not dose related or were within the normal range for this species.

TABLE 6 Selected clinical chemistry values for week 40

Parameter	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
Tot. Chol. (mg/dl)	67	110*	104*	115*	142*
GGT (U/L)	1	1	2	2	3*

* $p \geq 0.05$

c. Clinical Chemistries - Thyroid function

In addition to the above the following thyroid function tests were also performed at weeks 1, 14, 27, 40 and 53:
triiodothyronine (T3)
reverse T3 (rT3)
thyroxine (T4)
thyroid stimulating hormone (TSH)

Free T3 and free T3 were also determined at week 53.

Results - Attached table 6A from the study report presents the thyroid function data.

- TSH (5000 ppm) was statistically increased (%) at 1 and 14 weeks of the study. Subsequent time point values, although increased were not statistical.
- T4 and free T4 (5000 ppm) were only statistically depressed at 53 weeks although there was evidence of depression at other time points. T4 was also decreased ($p < 0.05$) in the 2500 ppm group at 40 and 53 weeks but not 3750 ppm. It was increased however in the 3750 ppm group at 27 weeks.
- Changes in T3, rT3 and free T3 did not appear to be related to treatment since they were sporadic with regard to dose or time, or were of a small magnitude.

6. Urinalysis - not performed

7. Sacrifice and Pathology

Fifteen rats selected at random were sacrificed at each of the following times:weeks 1, 14, 27, 40 and 53. The remainder were sacrificed at 104 weeks. All animals that died and that were sacrificed on schedule were subject to gross pathological examination. Livers were weighed following trimming and thyroid/parathyroids were trimmed and fixed prior to weighing. The following tissues were preserved in 10% neutral-buffered formalin, and processed for histologic examination:

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Thyroid/parathyroid
Liver
Gross lesions

a. Organ weight - As can be seen in table 7, absolute thyroid weight is consistently increased in males treated with 2500 ppm and above. Statistical significance however is apparent only at 14 and 26 weeks. Relative thyroid weights are statistically significant throughout most of the study at 3750 ppm and above.

TABLE 7 Organ weight - Absolute Thyroid (g)[a]

Week	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
1	.022	.020	.025	.024	.022
14	.026	.028	.030 (15%)	.032* (23%)	.032* (23%)
26	.035	.038 (9%)	.045* (28%)	.048* (37%)	.051* (46%)
40	.043	.049 (14%)	.049 (14%)	.050 (16%)	.050 (16%)
53	.050	.057 (14%)	.057 (14%)	.056 (12%)	.060 (20%)
104	.054	.056	.132[b]	.096[b]	.085[b]

* $p \geq 0.05$
a Does not include unscheduled deaths and moribund sacrifices.
b Standard deviations for these values were very large.

TABLE 8 Organ Weight - Relative Liver (to body weight)[1]

Week	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
1	3.609	4.036*	4.298*	4.410*	4.815*
14	2.529	2.950*	3.117*	3.262*	3.622*
26	2.452	2.800*	3.042*	3.431*	3.620*
40	2.362	2.728*	2.777*	3.084*	3.518*
53	2.487	2.647	3.048*	3.382*	3.472*
104	2.771	2.992*	3.460	3.789*	4.305*

* $p \geq 0.05$
1 Does not include unscheduled deaths and moribund sacrifices.

As can be seen in table 8, relative liver weight is increased in all groups at almost all time periods. Absolute liver weight is also statistically increased (8 - 55%) in a dose related manner in all groups throughout most of the study.

b. Gross pathology

Changes in thyroid gross pathology were first noted at the 14 week sacrifice and consisted of dark thyroid (2/15 rats) and enlarged thyroid (1/15 rats) at the high dose. By 27 weeks these observations had increased in frequency and occurred at 2500 ppm and above. Enlarged livers were also noted in these groups. These changes continued until term.

c. Microscopic pathology

Non-neoplastic lesions were observed in the thyroid and liver. In the thyroid there was a treatment related increase in follicular cell hypertrophy, hyperplasia and pigment, follicular cysts and a possible decreased colloid (see table 9).

TABLE 9 Select non-neoplastic thyroid follicular lesions (%)

Lesion	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
Hypertrophy	4	1	**8**	**17**	**34**
Hyperplasia	0	0	**0**	**2**	**2**
Pigment	53	54	**66**	**73**	**71**
Colloid decreased	2	**4**	**5**	**7**	**16**
Cysts	0	**3**	**3**	**8**	**5**
Colloid increased	1	1	1	**3**	**2**

N = 120, 116, 119, 120, 119 for controls to high dose (total animals examined for the study.

BOLD - most likely treatment related increase.

The following discussion of the individual thyroid follicular lesions is supported by table 12 at the end of this DER.

Hypertrophy increases occur as early as 1 week in the 2500 ppm (and above) group with a dose related response. Although the response appears to

temporarily diminish by week 26, there is a strong response by week 104 in the same treatment groups.

Hyperplasia increased only slightly at 3750 and 5000 ppm. Due to the low incidence it is difficult to characterize it as to time on study.

Pigment does not occur in any animals until week 26. At this time it occurred in all groups including controls. However, there was a marked increase at 3750 ppm and 5000 ppm (40%, 40%, 47%, 100% and 100% for controls to high dose). At week 40, the 2500 ppm treatment animals were also 100 % affected. By week 53 almost all animals in all groups had pigment in the thyroids (including controls).

Decreased colloid occurred sporadically throughout the study 7%, 33%, 33%, 33% and 80 % for controls to high dose). As the study progressed the incidence decreased and the lowest dose affected increased. By 53 weeks there were no cases.

Cysts did not occur in any control animals. Increases were present in all treated groups. The low dose animals were not affected until 53 weeks. They occurred sporadically in higher doses as early as 26 weeks, however.

Increased colloid was present in all groups and occurred sporadically throughout the study (1%, 1%, 1%, 3% and 2% for controls to high dose). It is difficult to determine if this effect is treatment related.

In the liver there was a treatment related increase in eosinophilic and basophilic foci of cellular alteration, hepatocellular enlargement and hepatocellular intracytoplasmic eosinophilic inclusions in groups at 2500 ppm and above. There was also an increase in periportal vacuolization at 3750 ppm and 5000 ppm (see table 10).

The incidence of **eosinophilic cellular** alteration is comparable among control and treated groups up to 52 weeks. At 104 weeks the incidence is increased at 2500 ppm and above (15, 22, 40, 67 and 40 % for control to high dose).

The incidence of **basophilic cellular alteration** is increased at 2500 ppm and above for unscheduled deaths and is also increased at 104 weeks.

Hepatocellular enlargement increased as early as 14 weeks in the 5000 ppm group, 26 weeks in the 3750 ppm group and 40 weeks in the 2500 ppm group. This lesion does not occur in the control and 1250 ppm groups.

The incidence of **hepatocellular intracytoplasmic** eosinophilic inclusions is observed as early as 26 weeks in the 3750 ppm and 5000 ppm groups and 40 weeks in the 2500 ppm group. This lesion is not observed in the 1250 ppm and controls.

By 14 weeks and clearly by 26 weeks, there appears to be a dose-related increase in the incidence of **periportal vacuolization** at 3750 ppm and above.

TABLE 10 Select non-neoplastic liver lesions in per cent

Lesion	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
Eosinophilic cellular alteration	6	3	**10**	**10**	**12**
Basophilic cellular alteration	6	9	**21**	**18**	**18**
Hepatocellular enlargement	0	0	**5**	**14**	**18**
Intracytoplasmic inclusions	0	0	**3**	**16**	**28**
Periportal vacuolization	2	0	0	**7**	**16**

N = 125, 124, 125, 125, 125 for controls to high dose (total animals examined for the study.
BOLD - most likely treatment related increase.

Neoplastic lesions (Thyroid follicular cell adenomas) are presented in table 11.

TABLE 11 Thyroid Tumors in Male Rats

	0 ppm	1250 ppm	2500 ppm	3750 ppm	5000 ppm
Follicular cell adenoma[1]	4/90(4)**	7/85(8)	7/88(8)	6/89(7)	15/89(17)**
Follicular cell carcinoma[2]	1/60(2)	1/54(2)	4/58(7)	3/59(5)	2/59(3)
Follicular cell tumors	5/90(6)**	8/85(9)	11/88(12)	9/89(10)	17/89(19)**

1 Denominator represents animals at risk, survivors after occurrence of first tumor.
Adenoma - first tumor ar week 27.
2 Carcinoma - first tumor at week 67.
Trend noted at controls, pair-wise comparison noted at dose group
** $p < 0.01$

The treatment related thyroid tumor response is limited to the 5000 ppm group and consists solely of follicular cell adenoma. This is consistent with an earlier rat oncogenicity study.

Historical control data are attached to this DER.

DISCUSSION

This was a well conducted and reported study. The treatment levels were mg/kg/day in the diet. Body weight gain was decreased greater than 10 % from 3750 ppm. The decrease in food consumption was minimal and probably not related to treatment.

Clinical chemistries (other than thyroid tests) that appeared to be affected by treatment included total cholesterol, increased at all dose levels and GGT, increased at 2500 ppm and above. The cholesterol increase is statistically significant, especially at the 5000 ppm dose. GGT is increased slightly from 2500 ppm and above and may be related to treatment.

The thyroid function test results were not as conclusive as those obtained in a 92 day study. One explanation for this is the age of the rat at initiation of treatment. The 92 day study started with 13 week old rats while those in this study were only 5 weeks old. TSH does however appear to be increased at 5000 ppm. Liver weight is increased at all doses and thyroid is also increased statistically from 2500 ppm and above. There is evidence of thyroid follicular effects (histologically) at all doses (decreased colloid and increased cysts). Pigment and hypertrophy of the follicular cells occurred at 2500 ppm. Hyperplasia was present to a limited extent only at 3750 and 5000 ppm. Thyroid follicular cell adenomas were increased only at 5000 ppm. Carcinomas were not increased. The earliest adenoma was observed at 26 weeks in the control group.

TABLE 12 Select Non-neoplastic Thyroid Follicular Lesions (%)

LESION	PPM Week	0	1250	2500	3750	5000
Hypertrophy	1	7	7	**27**	**40**	**93**
	14	7	0	0	**13**	**53**
	26	0	0	0	0	0
	40	0	0	0	0	**20**
	53	0	0	0	0	**7**
	104	8	0	**30**	**44**	**45**
	UD	5	8	0	**15**	**20**
Hyperplasia	1	0	0	0	0	0
	14	0	0	0	0	0
	26	0	0	0	0	0
	40	0	0	0	**7**	**7**
	53	0	0	0	0	0
	104	0	0	0	0	0
	UD	0	0	0	**4**	**4**
Pigment	1	0	0	0	0	0
	14	0	0	0	0	0
	26	40	40	47	**100**	**100**
	40	40	47	**100**	**100**	**100**
	53	93	100	100	100	100
	104	100	100	100	100	100
	UD	60	68	**80**	**89**	**80**
Colloid decreased	1	7	**33**	**33**	**33**	**80**
	14	7	0	**7**	**13**	**27**
	26	0	0	0	0	0
	40	0	0	0	**7**	**20**
	53	0	0	0	0	0
	104	0	0	0	0	0
	UD	0	0	0	0	0
Cysts	1	0	0	0	0	0
	14	0	0	0	0	0
	26	0	0	0	**7**	0
	40	0	0	0	**7**	**7**
	53	0	**7**	**7**	**13**	0
	104	0	0	**5**	**11**	**15**
	UD	0	**8**	**4**	**11**	**13**

UD - animals found dead of sacrifices moribund
N = 15 for weeks 1, 14, 26, 40 and 53
N = 26, 18, 20, 18, 20 for week 104 (control to high dose)
N = 19, 25, 24, 27, 25 for UD animals (control to high dose)
BOLD - most likely treatment related increase.

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PENDIMETHALIN

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