



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

7-24-92
013273

JUL 24 1992

FILE COPY

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Carcinogenicity Peer Review of Pendimethalin

FROM: William Greear, M.P.H. and *William B. Greear 6/17/92*
Marion Copley D.V.M., Section Head
Review Section IV, Toxicology Branch I *Marion Copley 7/1/92*
Health Effects Division (H7509C)

and
Esther Rinde, Ph.D. *E. Rinde*
Manager, Carcinogenicity Peer Review Committee
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Robert Taylor
Product Manager #25
Registration Division (H7505C)

The Health Effects Division Carcinogenicity Peer Review Committee met on 03/18/92 to discuss and evaluate the weight-of-the-evidence on pendimethalin with particular reference to its carcinogenic potential.

The Peer Review Committee agreed that pendimethalin should be classified as Group C - possible human carcinogen and recommended that for the purpose of risk characterization the Reference Dose (RfD) approach should be used for quantification of human risk.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Karl Baetcke

Karl D. Baetcke

Marcia Van Gemert

Marcia Van Gemert

Reto Engler

Reto Engler

Robert Beliles

Robert Beliles

Lucas Brennecke

Lucas H. Brennecke

William L. Burnam

William L. Burnam



Marion Copley

Marion Copley

George Ghali

G. Ghali

Richard Hill

Richard Hill

Hugh Pettigrew

Hugh Pettigrew

John Quest

John Quest

Yin-Tak Woo

Yin Tak Woo

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

William Greear¹William B. Greear 8/17/92

Bernice Fisher

Bernice Fisher

3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penelope Fenner-Crisp

Penelope A. Fenner-Crisp

Kerry Dearfield

Kerry Dearfield

Julie Du

Julie D. Du

Jean Parker

Jean Parker

Esther Rinde

Esther Rinde

William Sette

William Sette

4. Other Attendees: (Observers)

Eve Andersen (Clement)

Ann Clevenger (HED)

¹ Also a member of the PRC for this chemical. Signature indicates concurrence with the peer review unless otherwise noted.

B. Material Reviewed:

The material available for review consisted of DER's, one-liners, and other data summaries prepared by William Greear; tables and statistical analysis by Bernice Fisher. The material reviewed is attached to the file copy of this report. The data reviewed are based on studies submitted to the Agency by American Cyanamid.

C. Background Information:

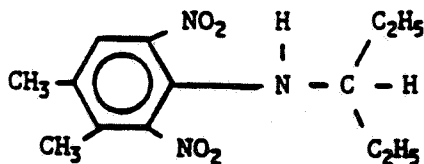
Pendimethalin, N-(1-ethylpropyl)-3,4-dimethyl-2,6 dinitrobenzenamine is a dinitroaniline herbicide registered for use on corn, rice, beans, peanuts, soybeans, cotton, sorghum, and sunflowers for the control of certain broadleaf weeds and grassy weed species. Pendimethalin is available as a technical material at 90% ai. It is also registered by the trade name PROWL®. End use formulations are a 1% and 10% granular and 2.98 lbs/gal, 3.0 lbs/gal, and 4.0 lbs/gal emulsifiable concentrate. The American Cyanamid Company produces pendimethalin. Following the Data Call-In Notice of the first Registration Standard of March 1985, a multigeneration study and two chronic studies in rodents were received.

The Caswell (or Tox Chem) Number of pendimethalin is 454BB.

The Chemical Abstracts Registry Number (CAS No.) is 40487-42-1.

The PC Number is 108501.

The structure of pendimethalin is



D. Evaluation of Carcinogenicity Evidence:

1. Rat Carcinogenicity Study No. 1

Reference: Weltman, R.H., "Chronic Dietary Toxicity and Oncogenicity Study in Rats Fed AC 92,553," April 20, 1987. MRID Number: 4010744-01, Study Number: HLA 6123112. Testing Facility: Hazleton Laboratories, Inc., Madison, WI.

a. Experimental Design

Technical pendimethalin (91.9% ai) was administered in the diet to groups of 55 male and 55 female Cr1:CD (SD)BR rats at 0 (control), 100, 500, or 5000 ppm (approximately 0, 5, 25 or 250 mg/kg/day) for 24 months. Additional groups of 10 animals/sex/dose were assigned to the 12-month interim sacrifice.

b. Discussion of Tumor and Hyperplasia Data

Both males and females had a significant, dose-related, increasing trend in thyroid follicular cell adenomas and a significant increase using pair wise comparisons between the controls and 5000 ppm group. (See Tables 1 and 2) Both males and females had a significantly increased trend for thyroid follicular cell hyperplasia, which was significant in pairwise comparisons at the high dose for females only. There was no statistically significant increase in carcinomas.

Table 1. Pendimethalin- Sprague-Dawley Male Rats (1987) Thyroid Follicular Cell Tumor and Hyperplasia Rates⁺ and Peto's Prevalence Test Results

| <u>Lesions</u> | <u>0</u> | Dose (ppm) | | |
|-------------------|----------|------------|------------------------|-------------------------|
| | | <u>100</u> | <u>500</u> | <u>5000</u> |
| hyperplasia (%) | 7/64(11) | 7/62(11) | 4/64(6) | 10 ^a /64(16) |
| p = | 0.028* | 0.421 | 0.870(n) | 0.121 |
| adenomas (%) | 3/64(5) | 2/62(3) | 3 ^b /64b(5) | 8/64(13) |
| p = | 0.003** | 0.720(n) | 0.491 | 0.038* |
| carcinomas (%) | 0/64(0) | 0/62(0) | 0/64(0) | 1/64 ^c (2) |
| p ⁺⁺ = | 0.252 | 1.000 | 1.000 | 0.500 |

^a First hyperplasia observed at week 53, dose 5000 ppm.

^b First adenoma observed at week 53, dose 500 ppm.

^c First carcinoma observed at week 93, dose 5000 ppm.

Table 2. Pendimethalin - Sprague-Dawley Female Rats (1987), Thyroid Follicular Cell Hyperplasia and Adenoma Tumor Rates⁺ and Peto's Prevalence Test Results

| <u>Lesions</u> | <u>0</u> | Dose (ppm) | | |
|-----------------|----------|------------|------------|------------------------|
| | | <u>100</u> | <u>500</u> | <u>5000</u> |
| Hyperplasia (%) | 1/34(13) | 1/44(2) | 3/37(8) | 8 ^a /45(18) |
| p= | 0.004** | 0.560(n) | 0.161 | 0.027* |
| Adenomas (%) | 1/41(2) | 1/49(2) | 1/43(2) | 7 ^b /53(13) |
| p= | 0.002** | 0.560 | 0.510 | 0.036* |

^a First hyperplasia observed at week 88, dose 5000 ppm.

^b First adenoma observed at week 53, dose 5000 ppm.

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

⁺⁺ Resultant p values based on application of Exact Trend test and Fisher's Exact test for pair-wise comparisons with control and each dose level.

Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level. If * then p<.05 and if ** then p<.01.

(n) = negative change from control.

The sponsor has submitted historical control data from 13 separate 2-year studies conducted from 1985 to 1990 at the testing laboratory, Hazleton-Wisconsin, Inc., in Sprague-Dawley rats. The incidence of adenomas in male (12.3%) and female (10.8%) rats in the 5000 ppm group exceeded the historical control ranges for males (0 to 8.1%; mean - 3.2%) and for females (0 to 5.7%; mean - 1.8%). The incidence of adenomas and carcinomas combined in the male 5000 ppm group (13.8%) exceeded the historical control range (0 to 8.1%, mean 3.3%).

c. Non-neoplastic Lesions

At the interim sacrifice, the thyroids of all 10 males and 10 females in the 5000 ppm group were diffusely dark. Most of the animals in the 5000 ppm group had diffusely darkened thyroids at terminal sacrifice. This was also observed in animals that were not sacrificed on schedule. A few animals in the 100 and 500 ppm group (approximately 3%) had diffusely dark thyroids.

The majority of the males and females in the 5000 ppm group had pigmentation of the follicular cells of the thyroid and discolored colloid in the thyroid. A few animals in the 500 ppm group had pigmentation of the follicular cells of the thyroid. There was an increase in follicular cell hyperplasia of the thyroid in males and females in the 5000 ppm group when compared to males and females in the control group. Follicular cell hyperplasia did not appear to be significantly increased in males and females in the 100 and 500 ppm groups (see Tables 1 and 2).

There was an increase in absolute and relative thyroid weight primarily in males (up to about 62% over controls) at 5000 ppm at the interim sacrifice. However, increased thyroid weights were not observed at terminal sacrifice.

The absolute and relative liver weights also were increased in both sexes at 5000 ppm. Increases were noted for GGT and total cholesterol at 5000 ppm.

There appeared to be a slight decrease in survival in the high dose males (36, 38, 42 and 29% for control through high dose). Statistical analysis showed a significant dose-related decreasing trend in survival in male rats.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The dosing was considered to be adequate for assessing the carcinogenic potential of pendimethalin. There were body weight gain depressions of 10.7% and 25.4% in males and females in the 5000 ppm group, respectively, at 13 weeks when compared to controls. Also, at the end of two years, body weight gain depressions in male and female rats in the 5000 ppm group were 29.7% and 15.8%, respectively, when compared to controls.

2. Rat Carcinogenicity Study No. 2

Reference: "Effects of Chronic Dietary Administration of AC 92,553 on the Function and Structure of Male Rat Thyroids," September 10, 1991. MRID Number 420478-02, Study Number: HLA 362-191, Testing Facility: Hazleton Laboratories America, Inc.

a. Experimental Design

Technical pendimethalin (92.6%) was administered in the diet to groups of 50 male Crl:CD(SD)BR rats at 0 (control), 1250, 2500, 3750 or 5000 ppm (approximate doses of 0, 51, 103, 154 or 213 mg/kg/day) for 24 months. Additional groups of 15 males/dose were sacrificed after receiving 1, 13, 26, 39 or 52 weeks of compound in the diet. Only males were tested.

b. Discussion of Tumor Data

The incidences of thyroid follicular cell adenomas and carcinomas in male rats are shown below.

Thyroid Follicular Cell Tumors and Hyperplasia in Male Rats (1990 study)

| <u>Lesion</u> | <u>Dose (ppm)</u> | | | | |
|-----------------|-------------------------|-------------|-------------|-----------------------|--------------------|
| | <u>0</u> | <u>1250</u> | <u>2500</u> | <u>3750</u> | <u>5000</u> |
| adenoma | 4 ^a /90(4)** | 7/85(8) | 7/88(8) | 6/89(7) | 15/89(17)** |
| carcinoma | 1/60(2) | 1/54(2) | 4/58(7) | 3 ^b /59(5) | 2/59(3) |
| combined tumors | 5/90(6)** | 8/85(9) | 11/88(12) | 9/89(10) | 17/89(19)** |
| hyperplasia | 0/90(0)* | 0/85(0) | 0/88(0) | 2/89 | 2 ^c /89 |

* Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.

^a first adenoma at week 27. ^b first carcinoma at week 67.

^c first hyperplasia at week 53

Significance of trend denoted at Control. Significance of pair-wise comparison with control denoted at Dose level.

* p < 0.05 ** p < 0.01

There was a significant trend ($p < 0.01$) for follicular cell adenomas and adenoma/carcinomas combined. Since the adenomas are responsible for significance in combined values, only adenomas will be discussed further. In addition, pairwise comparisons produced significant differences in adenomas between the control and 5000 ppm groups at the $p \leq 0.01$ level.

The incidence of adenomas in males in the 5000 ppm group (17%) exceeded the historical control range for males (0 to 8.1%, mean 3.2%).

c. Non-neoplastic Lesions

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 4). Absolute thyroid weights were increased from 57 to 144% in the 2500 ppm and up when compared to controls. This was seen as early as week 14 in the 3750 ppm group.

Table 4. 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.

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. Liver weight (relative) was increased in all groups treated with pendimethalin starting at week 1.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The body weights were statistically decreased at 2500 ppm and above when compared to controls. The decrease was greater than 10% during weeks 40-104.

3. Rat Chronic Feeding/Carcinogenicity Study No. 3

Reference: "A 24-month Oral Toxicity and Carcinogenicity Study of Compound AC 92,553 in Rats," August 21, 1974. MRID Number: 00059468, Study Number: 72R-746, Testing Facility: Bio/dynamics, Inc.

This study is "Invalid" and cannot be used in data analysis.

4. Mouse Carcinogenicity Study No. 1

Reference: Johnson, D.E., "Chronic Dietary Toxicity and Oncogenicity Study With AC 92,553 in Mice," October 5, 1988. MRID Number: 409099-01, Study Number: 141-028, Testing Facility: International Research and Development Corporation, Mattawan, MI.

a. Experimental Design

Pendimethalin technical was administered in the diet to groups of 55 male and 55 female CD-1 mice at 0 (control), 100, 500, or 5000 ppm (approximate doses, males - 0, 12.3, 62.3 or 622.1 mg/kg/day; females - 0, 15.6, 78.3 or 806.9 mg/kg/day) for 18 months. A second control group of 55 male and female mice each was included in the study. Additional groups of 10 mice/sex/dose were assigned to the 12-month sacrifice.

b. Discussion of Tumor Data

There were no increases in neoplasms reported for any dosed group.

c. Non-Neoplastic Lesions

Amyloidosis occurring in multiple tissues was increased in males and females in the 5000 ppm group. There was an increase in absolute and relative thyroid weight in females (33% and 24% over controls) and relative thyroid weight in males (9% over controls) treated at 5000ppm. Absolute and relative liver weights were also increased in all male treated groups and females in the 5000 ppm group.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

Adequate toxicity to test carcinogenic potential was achieved in females. Survival at 18 months was decreased in females in the 5000 ppm group (66%) when compared to controls (89%). Males and females in the 5000 ppm group exhibited increases in the liver/ gallbladder weight, liver/ gallbladder body weight ratio, and/or the liver/ gallbladder brain weight ratio at 12 months and at termination. On microscopic examination of the liver, no differences could be discerned among control and treated groups. The thyroid weight, thyroid body weight ratio, and/or thyroid brain weight ratio was increased in males and females in the 5000 ppm group. Organ weight, organ/body, and organ/brain weight ratio changes in male mice in the 5000 ppm group are not

considered to be adequate evidence to indicate that adequate toxicity was achieved. However, 5000 ppm is near the limit dose of 7000 ppm.

5. Mouse Chronic Feeding/Carcinogenicity Study No. 2

Reference: "An 18-month Carcinogenicity Study of AC 92,553 in Mice," April 2, 1974. MRID Number 00040301, Project Number: 71R-747, Testing Facility: Bio/dynamics, Inc.

This study is "Invalid" and can not be used in data analysis.

E. Additional Toxicology Data on Pendimethalin

1. Hormonal Mechanism Studies

a. A 92-day Thyroid Function Study in male rats, strain CD[Crl:CD(SD)] (HLA 6123-112, 8/5/91) was conducted at dose levels of 0, 100 or 5000 ppm (0, 4.98 or 245.4 mg/kg/day). A NOEL could not be determined. The LEL was 100 ppm based on decreases in T_3 and T_4 levels. In addition, at 5000 ppm there were: increases in TSH levels; decreases in body weight and body weight gain; increase in the incidence and severity of hypertrophy of thyroid follicular epithelial cells; and increases in absolute and relative thyroid weight.

TABLE 5 Levels of Serum TSH, T_3 and T_4 in Male Rats (% increase or decrease)

| Day 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) | 1.35* (-70.8) |

* p < 0.05

b. A 2-year Chronic Feeding Study² in male rats, strain Crl:CD(SD)BR (HLA 362-191, 9/10/91) was conducted at dose levels of 0, 1250, 2500, 3750 or 5000 ppm (0, 51, 103, 154 or 213 mg/kg/day). The systemic NOEL could not be determined. The LEL was less than or equal to 1250 ppm based on the finding of decreased colloid and an increase of cysts in the thyroid follicles as well as increased liver weight. The levels of T_3 and T_4 were erratic. The NOEL based on thyroid function was 3750 ppm (not definitive) due to an increase in TSH at 5000 ppm. In addition, at 2500 ppm there was an increase in pigment and hypertrophy of follicular cells. At 3750 ppm and above, there was also hyperplasia of follicular cells. Thyroid follicular adenomas, GGT and cholesterol were increased at 5000 ppm.

c. The registrant has submitted a draft report of a 2 week study indicating that the decreases in T_3 and T_4 were not due to decreased synthesis. Peer review committee did not review this study.

d. The registrant plans to conduct a study of thyroid hormone clearance to further elucidate the mechanism for depression of T_3 and T_4 .

2. Metabolism

When ¹⁴C-radiolabeled pendimethalin was administered to rats, about 70 percent of the radioactivity was excreted in the feces and 20 percent in the urine within 24 hours. The excretion of radioactivity in the urine peaked at 6 to 12 hours wherein 11.2 percent of the dose was excreted. In feces, the peak excretion interval was between 12 and 24 hours wherein 46.6 percent of the dose was excreted. The maximum residual radioactivity in the tissues was found in the 6-hour samples (except for fat at 12 hours). The levels of radioactivity detected in liver, kidney, muscle, fat, and blood at 6 hours were 29.8, 16.9, 1.3, 12.2, and 5.4 ppm, respectively. Within 96 hours, the radioactivity found in the tissues was 0.3 ppm or less, except for fat which was 0.9 ppm. The major portion of the radioactivity that was excreted in the feces was identified as the parent compound.

Pendimethalin is metabolized in rats mainly through oxidation of the 4-methyl group attached to the benzene ring as well as oxidation of the alkyl side chain of this N-substituted dinitroaniline compound (MRID No. 000446275, Study No. 2-463).

3. Mutagenicity

Pendimethalin has been tested in several mutagenicity studies. Acceptable tests fulfill all three categories for mutagenicity testing. The following studies have been conducted:

In Vitro Cytogenetics-CHO - Negative results were obtained when pendimethalin was tested at levels up to 25 μ g/plate without metabolic (S9) activation and

² This is the same study discussed in D.2.

100 ug/mL with metabolic activation (MRID No. 00153770, Study No. PH-320-AC-001-85). Acceptable.

DNA Repair (Unscheduled DNA synthesis) - Negative results were obtained when tested when pendimethalin was tested between 30 and 3000 ug/well (MRID No. 00153771, Study No. PH 311-AC-002-85). Acceptable.

Salmonella assay - Positive results were obtained in strains TA1538 (large increase) and TA98 (frame-shift mutations) with metabolic (S9) activation. No evidence of mutagenic activity was evident in strains TA1535, TA1537 and TA100. These results are based on several replicates. Dose levels ranged from 50 to 5000 ug/plate (MRID No. 00153768, Study No. 0166). Acceptable.

CHO/HGPRT Assay - Negative results were obtained at dose levels up to 80 ug/mL with metabolic (S9) activation. Inconclusive results, though suggestive increases, were obtained at levels up to 10 ug/mL without metabolic activation (MRID No. 00153769, Study No. PH-314-AC001-85). Unacceptable; need higher concentrations.

Host-Mediated Assay - Tested a prepared nitrosamine sample (C194269) found in the technical Prowl product. Negative results were obtained at dose levels up to 16.6 mg/mouse (MRID No. 00067519, Study No. N/A). Unacceptable; many questions, no toxicity.

Host-Mediated Assay - The question of the mutagenicity of pendimethalin (a process intermediate PROWL, 76.2% plus 15% nitrosamine) in this study has not been resolved. Dose levels used were 20.0 and 26.8 mg/mouse (MRID No. 00067519, Study No. N/A). Unacceptable.

Dominant Lethal - Pendimethalin was negative at the highest dose level tested of 2500 ppm (MRID No. 00026673, Study No. 2006). Unacceptable.

Salmonella/E. Coli assay - Pendimethalin was negative when tested at levels up to 1000 ug/disc or plate with and without metabolic activation (MRID No. 00067519, Study No. N/A). Unacceptable; strains unspecified.

The three acceptable tests meet the initial testing requirement for mutagenicity testing in the three categories of gene mutations, structural chromosomal aberrations and other genotoxic effects. The positive Salmonella results indicate that pendimethalin has genotoxic activity. An assay for germ cell effects or interaction is required to follow-up the Salmonella results.

Currently a mutagenicity study titled "Micronucleus Cytogenetic Assay in Mice with AC 92,553 Lab Study" No. T9801, June 7, 1991, D. L. Putman, M. J. Morris, Microbiological Associates, Inc., MRID #42027801 is under review. The results of this micronucleus test will not impact on the Salmonella follow-up requirement.

4. Developmental Toxicity

Pendimethalin did not produce developmental effects in rats, when given by gavage at doses up to 500 mg/kg (MRID No. 00025752, Study No. 362-155) or in rabbits at doses up to 60 mg/kg by gavage (MRID No. N/A, Study No. 362-164); however, maternal mortality and embryotoxicity (resorptions) were observed at 125 mg/kg in a rabbit pilot study (MRID No. N/A, Study No. 362-163).

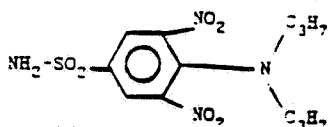
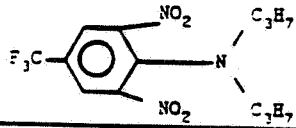
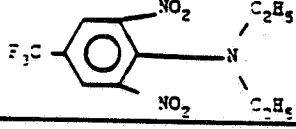
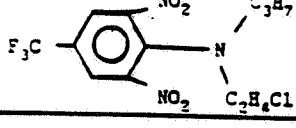
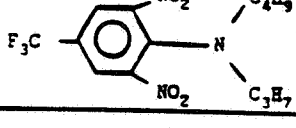
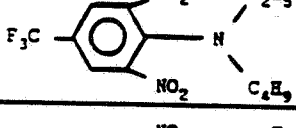
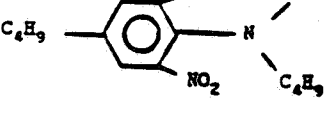
5. Structure-Activity Correlations

Pendimethalin is structurally related to oryzalin, trifluralin, benfluralin, fluchloralin, profluralin, ethalfluralin, and butralin. The primary difference between pendimethalin and the others is the methyl group present in pendimethalin as compared to the F_3C or $NH-SO_2$ group in most of the others. This difference limits the usefulness of the structure-activity comparisons.

Oryzalin was associated with increased mammary tumors (adenomas, fibroadenomas, and adenocarcinomas) in female F344 rats, thyroid follicular cell adenomas and carcinomas in male and female F344 rats, and skin tumors (fibromas and fibrosarcomas-males; papillomas, keratoacanthomas, and squamous cell sarcomas-males and females; and basal cell adenomas, preputial gland adenomas, sebaceous gland adenomas, Zymbal's gland adenomas and trichoepitheliomas-males and females) in F344 rats (MRID Nos. 00026779 and 00070569, Study Nos. R167 and R177). Oryzalin was categorized as a group "C" carcinogen with a Q^* of 1.3×10^{-1} (mg/kg/day) $^{-1}$ (based on the occurrence of mammary gland tumors (adenomas, fibroadenomas and adenocarcinomas combined) in female rats) by the HED Peer Review Committee. Trifluralin was associated with an increase in transitional cell carcinomas of the renal pelvis and thyroid follicular cell adenomas and carcinomas in male F344 rats, urinary tract tumors (transitional cell papillomas and carcinomas of the bladder) in female F344 rats (MRID No. 00044337, Study Nos. R-87 and R-97). Trifluralin was categorized as a group "C" carcinogen by the HED Peer Review Committee. The Q^* was calculated to be 7.7×10^{-3} (mg/kg/day) $^{-1}$. Ethalfluralin was associated with increased mammary gland tumors (fibroadenomas) in female Fischer 344 (MRID No. N/A, Study Nos. R267 and R277). Profluralin has been associated with increased liver tumors (hepatoma B) in male CD-1 mice (MRID No. N/A, Study No. 381-006). Ethalfluralin, benfluralin, fluchloralin, butralin and profluralin have not been examined by the HED Peer Review Committee.

Oryzalin produced sister chromatid exchanges following intraperitoneal administration to hamsters. Trifluralin was weakly positive in an Ames test, negative in two CHO tests, negative in one SCE test, but positive in another SCE test, and negative in a sex-linked recessive lethal test in *Drosophila*. Ethalfluralin was positive in two Ames tests with and without metabolic activation. It was also positive for the induction of chromosomal aberrations in an *in vitro* cytogenetics assay with CHO cells with metabolic activation. Butralin was positive in an Ames test with metabolic activation and in a mouse lymphoma assay with and without metabolic activation. (See Figure 1 for structurally related compounds.)

FIGURE 1. Structurally Related Compounds

| Structure | Name Tox Chem #/CAS # | Classification |
|---|---|---|
|  | Oryzalin (Surflan) 623A/19044-88-3 | Group C with Q* Rat + Thyroid, Mammary, Skin Mouse negative study |
|  | Trifluralin (Treflan) 889/1582-09-8 | Group C with Q* Rat + Kidney, Bladder, Thyroid Mouse negative study |
|  | Benfluralin (Benefin) 130/1861-40-1 | NA Rat inconclusive study Mouse inconclusive study |
|  | Fluchloralin (Basalin) 460B/33245-39-5 | NA Rat invalid study Mouse invalid study |
|  | Profluralin (Tolban) 271BB/26399-36-0 | NA Rat negative study Mouse + Hepatoma |
|  | Ethalfluralin (Sonalan) 453B/55283-68-6 | NA Rat + Mammary Mouse negative study |
|  | Butralin (Dibutalin) 125E/33629-47-9 | NA Rat negative study Mouse no studies |

+ Positive cancer study

NA Not applicable or not evaluated

6. Acute, Subchronic, and Chronic Toxicity Studies

The acute oral LD₅₀ of pendimethalin in male and female rats is 1250 mg/kg and 1050 mg/kg, respectively (Toxicity Category III). The acute dermal LD₅₀ in rabbits is greater than 5000 mg/kg (Toxicity Category III). The acute inhalation LC₅₀ for a 15 percent aqueous solution of the technical in rats was greater than 320 mg/L for a 4-hour exposure. Pendimethalin causes slight dermal and eye irritation in rabbits (Toxicity Category III) but is not a skin sensitizer. In a cataractogenicity study in chickens, the NOEL was determined to be greater than 3000 ppm.

Pendimethalin was administered to rats in a 90-day feeding study at dose levels of 0, 100, 500, and 5000 ppm in the diet. At the 5000 ppm dose level, there was a decrease in the hematocrit and hemoglobin in males, decreased body weight gain and food consumption, and hypertrophy of the liver accompanied by increased liver weights. The NOEL was 500 ppm. In a 90-day study in dogs, the NOEL was determined to be greater than 62.5 mg/kg/day (ASALIS) when pendimethalin was administered by gavage. In a 21-day dermal study in rabbits, the NOEL was determined to be greater than 1000 mg/kg. In a 30 day feeding study in mice, the NOEL was determined to be greater than 2000 ppm (ASALIS).

Although not discussed at the PR meeting, the HED file noted that the NOEL for the 2-year dog study (#20755, MRID 00067519) was not 12.5 mg/kg/day and the LEL was 50 mg/kg/day based on increases in serum alkaline phosphatase, increased liver weight and hepatic lesions.

A three-generation reproduction study was conducted in rats using dose levels of 500 and 5000 ppm pendimethalin in the diet. The NOEL was determined to be 500 ppm based on reduced litter size and pup body weight and a decrease in the survival index.

F. Weight of Evidence Considerations

The Committee considered the following facts regarding the toxicology data on pendimethalin in a weight-of-the-evidence determination of carcinogenic potential:

1. There is positive evidence for benign thyroid tumors in rats. In rat study No. 1, pendimethalin was associated with a statistically significant increased trend and pairwise comparison at 5000 ppm for thyroid follicular cell adenomas in both male and female Sprague-Dawley rats. In rat study No. 2, there was also a statistically significant increased trend and pairwise comparison at 5000 ppm for thyroid follicular cell adenomas, but only male rats were tested. Thyroid follicular cell hyperplasia showed positive dose trends in the two studies.
2. The incidence of thyroid follicular cell adenomas and carcinomas in males and adenomas in females was outside the range reported for historical controls at the testing laboratory for all 13 studies.
3. The dosing was adequate for assessing carcinogenic potential, but the dose in rat study No. 1 may have been excessive, as indicated by the increased mortality in males.
4. Pendimethalin was not associated with increases in neoplasms when fed to CD-1 mice at doses up to 5000 ppm. The study appeared to have been adequately conducted.
5. Pendimethalin was positive in a Salmonella assay for frame shift mutations. Negative results were found in a structural chromosomal aberration test (in vitro cytogenetic CHO assay), and in a DNA repair (UDS) assay.
6. The thyroid follicular cell tumor response is also seen in two other members of this class of compounds. Oryzalin was associated with thyroid follicular cell tumors (adenomas and carcinomas) in male and female F344 rats. Trifluralin was associated with thyroid follicular cell tumors (adenomas) in male F344 rats. However, these are structural features of these analogs that lead one to think they may not be predictors of this chemical's behavior.
7. The PRC determined that there is some evidence that the thyroid tumors could be attributed to a disruption of the thyroid-pituitary hormonal balance. In a 92-day feeding study in male rats, there was evidence of a hormonal effect on the thyroid that included decreases in T_3 and T_4 at 100 and 5000 ppm and a marked increase in TSH at 5000 ppm. There was evidence for goitrogenic activity in vivo since there was follicular cell hypertrophy, increased pigmentation, decreased colloid, and increased thyroid weight. Also, there was evidence of progression from hypertrophy to hyperplasia and adenomas.

However, the PRC determined that there was insufficient evidence to conclude with certainty if the neoplasms observed were necessarily due to thyroid-pituitary imbalance and if so, by what particular mechanism. The PRC suggests that the following types of tests will fill this data gap:

tests to determine specific evidence for reduced hormone synthesis or increased clearance of T_4 from the serum

tests to determine whether the thyroid effects are reversible

tests to determine whether iodine carrier proteins are interrupted

tests to determine whether increased biliary excretion of T_4 may have lead to the decreased T_4 levels.

tests to determine whether glucuronide conjugates in the liver are involved, as indicated by UDP-glucuronosyltransferase activity levels

tests to determine whether liver enzyme induction mechanisms are involved, as evidenced by histological changes

The PRC also recommended:

a comparative DNA binding test, which would only be useful if similar tests with trifluralin are positive

tests to examine the possible role of genotoxicity, especially in light of the strong Salmonella result (TA 1538, TA98)

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for pendimethalin should be Group C - possible human carcinogen and recommended that for the purpose of risk characterization the Reference Dose (RfD) approach should be used for quantification of human risk .

This decision was based on the statistically significant increased trend and pairwise comparison between the high dose group and controls for thyroid follicular cell adenomas in male and female rats. This study was conducted using adequate doses for the determination of carcinogenic activity. Pendimethalin induces gene mutations, but not aberrations or DNA damage/repair based on acceptable studies. Structurally related compounds showed evidence of tumorigenic activity.

The PRC was requested to consider the possibility of using the threshold model for thyroid neoplasms for pendimethalin (see Appendix). While it was suggestive, the evidence was not sufficient to support hormonal mechanisms for thyroid neoplasms.

REFERENCES FOR LIVER-INDUCED EFFECTS ON THYROID-PITUITARY STATUS

Brown, C.G. et al. 1987. Effects of toxic doses of a novel histamine (H₂) antagonist on the rat thyroid. *Fd. Chem. Toxicol* 25:787-794.

Canan, P.G. and DeGrout, L.J. 1991. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocrin? Rev.*

Comer, C.P. et al. 1985. Changes in thyroid function and liver UDP glucuronosyl transferase activity in rats following a novel imidazole. *Toxicol Appl Pharmacol* 80:427-436.

McClain, R.M. et al. 1989. The effect of phenobarbital on the metabolism and excretion of thyroxine in rats. *Toxicol Appl Pharmacol* 98:216-228.

Senler, D.E. et al. 1989. The effects of chronic ingestion of spironolactone on seven thyrotropin and thyroid hormones in the male rat. *Toxicol Appl Pharmacol* 98:263-268.

APPENDIX

Taken from the Amitrole Draft Peer Review Document
(Rinde to Yowell 11/20/89)
and adapted by William Greear for pendimethalin.

The following guidance is given in the Agency's DRAFT Policy Document (Thyroid Follicular Carcinogenesis: Mechanistic and Science Policy Considerations, SAB Review Draft, May 1988):

"Studies over the last several decades in multiple laboratories and using a number of different treatment regimens (eg., iodine deficiency) have demonstrated the significance of long-term thyroid-pituitary hormonal imbalance in thyroid carcinogenesis. A consistent progression of events is noted: reduction in thyroid hormone concentrations, elevation in TSH levels, cellular hypertrophy and hyperplasia, nodular hyperplasia, and neoplasia. Hyperplasia and sometimes neoplasia of the pituitary may also be seen. A block in any of the early steps acts as a block for subsequent steps including tumor development, and cessation of treatment at an early stage in the progression results in regression toward normal thyroid structure and function. Based on these observations the Agency concludes that:

- a. thyroid follicular cell tumors may arise from long-term disturbances in thyroid-pituitary feedback under conditions of reduced circulating thyroid hormone and elevated TSH levels:
- b. the steps leading to these tumors are expected to show thresholds, such that the risks of tumor development are minimal when thyroid-pituitary homeostasis exists; and
- c. models that assume thresholds may be used to assess the risks of thyroid follicular cell tumors where there is evidence of thyroid-pituitary hormonal imbalance."

Two basic questions must be addressed before this policy is applied.

"The first is a qualitative issue which addresses whether it is reasonable to presume that the neoplasms are due to thyroid-pituitary imbalance. A corollary issue is the extent to which other carcinogenic mechanisms can be discounted. The second question concerns the procedures to be employed in estimating the risks of these agents."

"The answers to the first question allow one to assign chemicals producing thyroid tumors to one of three categories. The assignation is based upon knowledge as to whether the chemical disrupts thyroid-pituitary feedback, whether tumors other than thyroid follicular cell (and relevant pituitary) tumors are found, and whether mechanisms other than thyroid-pituitary imbalance may apply to the observed tumor response."

2. Determination of whether neoplasms are due to thyroid-pituitary imbalance

The document goes on to describe the three factors which should be considered in making this determination (answering the first question, or "qualitative issue"). These are addressed as they apply to Pendimethalin follows:

FACTOR I. Consideration of whether the thyroid tumors associated with administration of Pendimethalin can be attributed to disruption of the thyroid-pituitary hormonal balance. (In addressing this factor, the Policy states, six indicators should be considered.)

a. Goitrogenic activity in vivo:

Thyroid follicular cell hypertrophy was observed in males (only sex tested) in the 92-day thyroid function study and in the 2-year rat study no. 2. In the 2-year rat study no. 1 there was increased pigmentation of the follicular cells and discolored colloid of the thyroid in males and females. There was decreased colloid in the follicles in males (only sex tested) in the 2-year rat study no. 2. Thyroid follicular cell hyperplasia was observed in rats in the 2-year chronic/carcinogenicity feeding study no. 1 (both sexes) and study no. 2. There was a dose-related increase in absolute and relative thyroid weight in males (only sex tested) in the 92-day hormonal mechanism study. In both 2-year rat studies (no. 1 and 2) there was also increased absolute and/or relative thyroid weight (males and females when tested). There were also significant increases in the absolute and/or relative thyroid weight in the chronic/carcinogenicity mouse study no. 1.

b. Clinical chemistry changes (eg., reduced thyroid hormone and increased TSH serum concentrations):

In the 92-day hormonal mechanism study, T_3 and T_4 were significantly elevated in males (only sex tested) and TSH was significantly decreased. In the 2-year rat study no. 2 there was an increase in TSH in males (only sex tested) but T_3 and T_4 levels were quite variable.

c. Specific evidence of reduced hormone synthesis (eg., inhibited iodine uptake) or increased thyroid hormone clearance (eg., enhanced biliary excretion):

No information is available other than a reported decrease in colloid in the follicles in males (only sex tested) in the 2-year rat study no. 2.

- d. Evidence of progression (eg., hypertrophy/hyperplasia, nodular hyperplasia - neoplasia):

There is possible evidence of progression in both 2-year rat studies based on increases in hypertrophy and/or hyperplasia and adenomas of the thyroid follicular cells. There is no evidence of progression to malignancy. Only hypertrophy was apparent in the 92-day rat study.

- e. Reversibility of lesions after exposure is terminated:

There is no information.

- f. SAR to other thyroid tumorigens:

It is structurally related to Trifluralin and Oryzalin with reservations noted in the SAR section of this document.

Based on the overall judgment of the six indicators in Factor I, it may be concluded that there is suggestive evidence that the thyroid tumors in the rat associated with administration of Pendimethalin may be due to a disruption in the thyroid-pituitary status.

FACTOR II. Consideration of the extent to which genotoxicity may account for the observed tumor effects.

The mutagenicity data on Pendimethalin are equivocal. There are some possible indications of mutagenic activity in the point mutation tests (frame shift). Although one host mediated assay was negative a second test is being questioned. A second Ames test, HGPRT (CHO), dominant lethal, in vitro cytogenetics (CHO) and DNA repair are negative.

FACTOR III. Evaluation of neoplasms in addition to thyroid follicular tumors, including pituitary tumors.

No other treatment-related neoplastic lesions were observed in any study.

Conclusions: As indicated above, based on the overall judgment of the six indicators in Factor I and III, it may be concluded that there is suggestive evidence that the thyroid tumors in the rat associated with administration of Pendimethalin may be due to a disruption in the thyroid-pituitary status. Factors II is equivocal.

3. Factors to be Considered in Determining Method to be Used in Estimating the Risks of Pendimethalin

Again, this guidance is taken from the Amitrole Peer Review Document and revised for Pendimethalin. The Committee is requested to consider these points when determining which method is to be used for estimating the carcinogenic risk for Pendimethalin.

Guidance given in the EPA DRAFT policy on Thyroid Neoplasia for proceeding with the quantitation of risk is as follows:

- a. "Threshold considerations should be applied in dose-response assessments for those chemical substances where (1) only thyroid tumors (and relevant pituitary tumors) have been produced; (2) the tumors can be attributed to a disruption in thyroid-pituitary hormonal homeostasis; and (3) potential mechanisms other than thyroid-pituitary imbalance (eg., genotoxicity) can be disregarded.
- b. Special attention should be given to chemicals (1) that have induced thyroid tumors (and relevant pituitary tumors) that may be due to thyroid-pituitary imbalance, and (2) where there is also evidence of either a genotoxic potential or the induction of neoplasms at sites other than the thyroid (or pituitary). Generally, those cases will be approached using various principles laid out in the EPA Guidelines for Carcinogen Risk Assessment. A strong rationale must be articulated for handling these agents otherwise.
- c. For those chemicals producing thyroid tumors that do not seem to be acting via thyroid-pituitary hormonal inhibition, dose-response assessments will be performed in accordance with the EPA Guidelines for Carcinogen Risk Assessment."