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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

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

SUBJECT: PP#'S 3F02788, 3F02792, 3F02844, 4F03042, 4F03121 AND 8F03655
Request to Reevaluate the Carcinogenic Potential of Pendimethalin (EPA ID#285624), and Reevaluation of Developmental Toxicity Studies

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FROM: William B. Greear, M.P.H. William B. Greear 1/24/96
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THRU: John D. Doherty, Ph.D., Acting Section Head
Review Section IV, Toxicology Branch I
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John D. Doherty Section Head 2/2/96

I. CONCLUSIONS:

The new submission from American Cyanamid contains several studies that indicate that the production of thyroid follicular cell tumors in rats can probably be attributed to the disruption of the thyroid-pituitary hormonal balance.

Additionally the previously submitted rat and rabbit developmental toxicity studies have been reevaluated at the request of the RfD/Peer Review committee. It was concluded that:

"The rat developmental toxicity study is supplementary and, thus, does not satisfy the guideline requirement (83-3). However, this study in conjunction with the rabbit developmental toxicity study can be used to satisfy the guideline requirement (83-3). In order that the rabbit developmental toxicity be considered to be acceptable, individual litter data (fetal alterations) and historical control data must be submitted. However, if these data are not submitted, a confirmatory developmental toxicity study in rabbits will have to be conducted."

II. REQUESTED ACTION:

Under a cover letter dated February 1994, Zareen Ahmed, of the American Cyanamid Company, has submitted the following studies for evaluation and reconsideration by HED's Peer Review Committee on Carcinogenesis in arriving at the appropriate Category for carcinogens. It is American Cyanamid's belief that pendimethalin should be classified as a Category D (not classifiable as to human carcinogenicity) carcinogen rather than a Category C (possible human carcinogen). The new studies were submitted to demonstrate that the formation of follicular cell tumors of the rat thyroid was induced by a secondary mechanism resulting from administration of excessively high dietary levels of pendimethalin.

Studies Submitted (February 14, 1994)

- 56-Day Thyroid Function Study in Albino Rats with AC 92,553; American Cyanamid Company Study No. L-2366; Toxicology Report No. AX 93-1; May 28 1993, (MRID 43135001).

- 92-Day Thyroid Function Study in Albino Rats with AC 92,553; American Cyanamid Company Study No. T-0270; Toxicology Report No. AX 91-1: August 5, 1991, (MRID 43135002). (This study was submitted on September 16, 1991 and reviewed by the Peer Review Committee, (Page 9 of the Committee Report), but no MRID No. was received. It is being resubmitted just in order to obtain a MRID No.).

- 14-Day Intrathyroidal Metabolism Study in Male Rats with AC 92,553; University of Massachusetts Medical School, Division of Endocrinology, Experiment No. UM-92-03-01; American Cyanamid Company Protocol No. 971-92-107; April 16, 1993, (MRID 43135003).

- Effect of Ingestion of AC92,553 on Biliary Excretion and Hepatic Metabolism of Thyroxine, University of Massachusetts Medical School, Division of Endocrinology, Experiment No. UM-92-03-01; American

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Cyanamid Company Protocol No. 971-92-107; April 16, 1993, (MRID 43135004).

- AC 92,553 Lot No. AC 5213-72A): Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) and Escherichia coli WP2 urvA Reverse Mutation Assay with a Confirmatory Assay. Microbiological Associates, Inc., Study No. TD847.501114; American Cyanamid Company Protocol No. 971-93-117; December 3, 1993, (MRID 43177801).

- Bacterial/Microsome Reverse Mutation (Ames) Test on AC 92,553 (Lot No. AC 5042-52F); American Cyanamid Company Report GTOX Vol. 5, No. 6; March 3, 1988; April 26, 1993 (reformatted) Protocol No. 971-92-107; April 16, 1993, (MRID 43135005).

- AC 92,553: Salmonella/Mammalian-Microsome Plate Incorporation Assay (Ames Test) and Escherichia coli WP2 urvA Reverse Mutation Assay with a Confirmatory Assay. Microbiological Associates, Inc., Study No. TC892.501114; American Cyanamid Company Protocol No. 971-93-101; May 5, 1993, (MRID 43135006).

- AC 92,553: Detection of Single Strand Breaks, DNA/DNA and DNA/Protein Cross Links in Rat Testicular DNA by Alkaline Elution. Microbiological Associates, Inc., Study No. TC724.396; American Cyanamid Company Protocol No. 971-92-124; May 5, 1993, MRID 43135007).

- CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay. AC 92, 553 (Lot 5042-37D). Study PH-314-AC-002-85. Pharmakon Research International, Waverly, PA. October 26, 1985; April 23, 1993 (reformatted), (MRID 43177802).

Previously Submitted Studies Requiring Reevaluation

- **Teratology Study in Rats: AC 92,553 Final Report. Report No. 362-155. Hazleton Laboratories America, Inc. Vienna, VA. August 17, 1979. (MRID 00025752).**

- **Teratology Study in Rabbits: AC 92,553 Final Report. Report No. 362-163. Hazleton Laboratories America. Inc. Vienna, VA. May 11, 1981. (MRID 00117444).**

III. RESULTS:

A. Thyroid Carcinogenesis (New Studies)

o 56-Day Thyroid Function Study (MRID #431350-01)

In a special 56-day feeding study to determine thyroid function (MRID 43135001), groups of 65-70 (5-15 per sacrifice time) male Crl:CD(SD) rats were treated at dose levels of 0, 500 or 5000 ppm (0, 31 or 292 mg/kg/day) of AC 92,553 (pendimethalin, 92.6%, Lot #AC5213-72A) 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%) and increased percent free T_3 (13%), 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 to transthyretrin binding; increased percent free T_4 (117%), percent free T_3 (26%) and $[^{125}I]$-T 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. **The LOEL was 500 ppm (31 mg/kg/day based on thyroid effects. The NOEL could not be determined.**

The study is **Core-Supplementary** and does not by itself satisfy the guideline requirement for a series 85-1 metabolism study.

o 14-Day Intrathyroidal Metabolism Study (MRID 431350-03)

In a special 14-day feeding study to determine thyroid function (MRID 43135003), AC 92,553 (Pendimethalin, 92.6%, Lot #AC5213-72A) was administered in the diet to groups of 10 male Crl:CD(SD) rats at dose levels of 0, 100 or 5000 ppm (corresponding to 0, 10 or 500 mg/kg/day).

At 5000 ppm AC 92,533 for 14 days, TSH was increased and T_4 and T_3 were decreased. No treatment related effects were observed for rT_3 levels, thyroid weight, ^{131}I uptake in MIT, DIT or T_4.

There was a significant increase of ^{131}I uptake by the thyroid of rats in the 5000 ppm group and an increase in incorporation of ^{131}I in T_3. Total T_3 and T_4 levels in the thyroid were not affected by treatment at 5000 ppm. **The LOEL is 5,000 ppm (500 mg/kg/day) based on thyroid effects. The NOEL is 100 ppm (10 mg/kg/day).**

The study is **Core-Supplementary** and does not by itself satisfy the guideline requirement for a series 85-1 metabolism study.

o Biliary Excretion and Hepatic Metabolism (MRID #431350-04)

In a second special 14-day feeding study to determine biliary excretion and hepatic metabolism, AC 92,553 (pendimethalin, 92.98%, Lot #AC6539-77A) was administered to groups of 10 male Crl:CD(SD) rats at dose levels of 0, 100 or 5,000 ppm (corresponding to 0, 10 or 500 mg/kg/day).

Ingestion of 5000 ppm produced decreases in serum T_3 and T_4 with a compensatory increase in TSH. Also increased were liver weight, bile flow and cumulative biliary excretion of ^{125}I-T_4 with a slight increase in T_4-glucuronytransferase activity detected by generation of ^{125}I-T_4 glucuronide from ^{125}I-T_4 *in vitro* by hepatic microsomes. The increase in enzyme activity was also demonstrated *in vivo* by a significant increase in biliary excretion of ^{125}I-T4-glucuronide. **The LOEL is 5,000 ppm (500 mg/kg/day) based on thyroid effects. The NOEL is 100 ppm (10 mg/kg/day).**

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

o CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay (MRID 43177802)

In a forward mutation study at the HGPRT locus in Chinese hamster ovary CHO-K1-BH4 cells in culture, cells were exposed to Pendimethalin at concentrations of 1, 5, 7.5, 10, 20, 30, 40 and 50 µg/ml in the absence of an exogenous metabolic activation system and to 10, 25, 50, 75, 100, 125, 150 and 175 µg/ml in the presence of an exogenous metabolic activation system (S9-mix). Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat liver. The test material was delivered in DMSO.

Cytotoxicity was unacceptably high at 30, 40 and 50 µg/ml in the absence of S9 mix and at 125, 150 and 175 µg/ml with S9 mix; therefore, mutagenicity was not evaluated at these concentrations. A yellow precipitate was seen at concentrations ≥50 µg/ml. Positive, negative and vehicle control values were appropriate. **There was no evidence of induced mutant colonies**

over background either with or without S9 mix at any concentration evaluated in this study.

This study is classified as an acceptable study. It satisfies the guideline requirement for an in vitro mammalian cell gene mutation assay.

- *Salmonella*/Mammalian Activation Gene Mutation Assay and *Escherichia coli* WP2(uvrA) Reverse Gene Mutation Assay (84-2(a)) (MRID 43135006)

In a Salmonella/microsome plate incorporation assay and in an *Escherichia coli* WP2(uvrA) reverse mutation assay (MRID #431350-06), strains TA98, TA100, TA1535, TA1537, TA1538 and WP2(uvrA) were exposed to Pendimethalin at concentrations of 50, 100, 250, 500 and 750 µg/plate without exogenous metabolic activation and to the same concentrations plus an additional concentration of 25 µg/plate with exogenous metabolic activation. A confirmatory assay tested concentrations of 25, 50, 100, 250, 500 and 750 µg/plate both with and without S9 mix. Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat livers. The test material was delivered in DMSO.

No cytotoxicity was seen at any concentration tested up to 5000 µg/plate. The upper concentration tested was limited by solubility of the test material. Positive and vehicle control values were appropriate. **No evidence of a mutagenic response was seen at any dose in any strain with or without S9 mix in either assay.**

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (84-2(a))

- Salmonella/Mammalian Activation Gene Mutation Assay (MRID 43135005)

In a *Salmonella*/microsome plate incorporation and disk assay and in an *Escherichia coli* WP2(uvrA) reverse mutation assay (MRID #431350-05), strains TA98, TA100, TA1535, TA1537, and WP2(uvrA) were exposed to Pendimethalin at concentrations of 50, 158, 500, 1581 and 5000 µg/plate or 1000 µg/paper disk/plate, with and without exogenous metabolic activation. Preparations for metabolic activation were made from Aroclor 1254 induced male rat livers. The test material was delivered in DMSO.

Cytotoxicity determinations were not made or discussed in this study. The highest concentration was limited by solubility (a precipitate was seen at 1581 and 5000 µg/plate). Positive and vehicle controls were appropriate. **There was no evidence of**

induced mutant colonies over background vehicle control values at any concentration of Pendimethalin tested in any strain with or without S9 mix.

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (84-2(a))

o *Salmonella*/Mammalian Activation Gene Mutation Assay and *Escherichia coli* WP2(uvrA) Reverse Gene Mutation Assay (MRID 43177801)

In a Salmonella/microsome plate incorporation assay and in an *Escherichia coli* WP2(uvrA) reverse mutation assay (MRID #431778-01), strains TA98, TA100, TA1535, TA1537, TA1538 and WP2(uvrA) were exposed to Pendimethalin at concentrations of 25, 50, 100, 250, 500 and 750 µg/plate, with and without exogenous metabolic activation. Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat livers. The test material was delivered in DMSO.

No cytotoxicity was seen at any concentration of Pendimethalin tested. The upper concentration was limited by test material solubility (a precipitate was observed at concentrations of 750 µg/plate and above). Positive and vehicle control values were appropriate. **There was no evidence of an increase number of mutant colonies over solvent control values at any concentration of Pendimethalin tested, either with or without S9 mix.**

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (84-2(a))

o In vitro Mammalian Cytogenetics Assay in Chinese Hamster Ovary CHO-K1-BH4 Cells in Culture (MRID 43177802)

In a forward mutation study at the HGPRT locus in Chinese hamster ovary CHO-K1-BH4 cells in culture, cells were exposed to Pendimethalin at concentrations of 1, 5, 7.5, 10, 20, 30, 40 and 50 µg/ml in the absence of an exogenous metabolic activation system and to 10, 25, 50, 75, 100, 125, 150 and 175 µg/ml in the presence of an exogenous metabolic activation system (S9-mix). Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat liver. The test material was delivered in DMSO.

Cytotoxicity was unacceptably high at 30, 40 and 50 µg/ml in the absence of S9 mix and at 125, 150 and 175 µg/ml with S9 mix; therefore, mutagenicity was not evaluated at these concentrations. A yellow precipitate was seen at concentrations

≥50 µg/ml. Positive, negative and vehicle control values were appropriate. **There was no evidence of induced mutant colonies over background either with or without S9 mix at any concentration evaluated in this study.**

This study is classified as an acceptable study. It satisfies the guideline requirement for an in vitro mammalian cell gene mutation assay.

B. Developmental Toxicity (Previously Submitted Studies)

o Oral Teratology Study in Rats: AC 92,553 Final Report (MRID 00025752

Pendimethalin (94.2% a.i.) was administered in corn oil to groups of 30 mated Sprague-Dawley CD strain rats by gavage at daily dose levels of 0, 125, 250, or 500 mg/kg/day from gestation day 6 through 15 (MRID 00025752). Females were observed for signs of toxicity, and body weights were measured during gestation. Animals were sacrificed on gestation day 21 and reproductive observations were made and uteri were examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

There were no maternal or developmental effects noted at any dose level tested, and based on these results, the **NOELs for developmental and maternal toxicity are ≥500 mg/kg/day (highest dose tested).**

This study should be classified as Supplementary and used in conjunction with the rabbit developmental toxicity study (MRID 00117444) to satisfy guideline requirement §83-3. It is not upgradable because an adequate dose range may not have been tested.

o Teratology Study in Rabbits: AC 92,553 Final Report (MRID 00117444)

Pendimethalin was administered in corn oil to groups of 20 artificially inseminated New Zealand White strain rabbits by gavage at dose levels of 0, 15, 30, or 60 mg/kg/day from gestation day 6 through 18 (MRID 00117444). Females were observed for signs of toxicity, and body weights were measured during gestation. Animals were sacrificed on gestation day 29 and reproductive observations were made and uteri were examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

No maternal toxicity was reported at doses ≤60 mg/kg/day (highest dose tested). However, the range-finding study

indicated that doses ≥ 125 mg/kg/day were associated with increased mortality (3/5, 5/5 and 4/5 in the 125, 250, and 500 mg/kg/day, respectively compared with 0/5 in the control group). A slight increase in the mean incidence of skeletal anomalies in the mid- and high-dose groups which consisted of findings of less than twelve pairs of ribs (0/111, 1/118 and 4/107 fetuses in the control, mid-, and high-dose groups, respectively, not statistically significant) and/or missing or incompletely ossified vertebrae (0/111, 1/118 and 7/107 fetuses in the control, mid and high dose groups, respectively). No individual litter data or historical control data were available in the report to support a conclusion regarding the significance of these alterations. **A developmental toxicity NOEL could not be determined from this study.**

This study does not satisfy §83-3 guideline requirements for a rabbit developmental toxicity study and should be classified as Supplementary. It is upgradable pending receipt of individual litter data (fetal alterations) and historical control data.

IV. DISCUSSION

A. Mutagenicity

HED indicated in the Cancer Peer Review Document for pendimethalin that "...three acceptable tests meet the initial testing requirements 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." The Sponsor has supplied studies in germ cells, the CHO/HGPRT assay and the alkaline elution assay. The tests were negative for genotoxicity in mammalian cells. The alkaline elution assay specifically tests for possible frame-shift mutations and base-pair substitutions. There is only minor concern with respect to the potential mutagenicity of pendimethalin based on the negative results obtained in these tests together with the negative results obtained in a battery of mutagenicity tests (which included 2 repeat negative tests in Salmonella).

B. Thyroid Carcinogenesis

In the Cancer Peer Review document on the potential carcinogenicity of pendimethalin, the Committee provided three factors that must be addressed in order to determine whether the thyroid tumors associated with administration of pendimethalin could be attributed to disruption of the thyroid-pituitary hormonal balance. These factors are discussed below with respect to pendimethalin.

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 an/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.

In a newly submitted 56-day thyroid function study (only males tested), there were increased absolute and relative thyroid weights. There were histologic changes in the thyroid: increased follicular cell height, the area of the follicles occupied by colloid was decreased and the diameter of the thyroid follicles was decreased. The ultrastructural changes (numerous distended rough endoplastic reticulum, prominent Golgi apparatuses associated with small granules, numerous colloid droplets and large mitochondria) in the thyroid were stated to be indicative of a mild to moderate TSH stimulation. The majority of these parameter tended to be reversible after the 28 day recovery period.

b. Clinical chemistry changes (e.g., 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.

In the 56-day thyroid function study, T_3 and T_4 were decreased during the 28-day treatment period. In a 14-day intrathyroidal metabolism study TSH was increased and T_3 and T_4 were decreased.

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

In the 14-day intra thyroidal metabolism study, there were decreases in T_3 and T_4, and increases in TSH and ^{131}I uptake by the thyroid. Pendimethalin did not affect the organofication of ^{131}I or the percentage of ^{131}I incorporated into T_4, monoiodotyrosine (MIT) or diiodotyrosine (DIT). Pendimethalin, therefore, does not appear to affect the synthesis of thyroid hormones or iodine metabolism. Pendimethalin is not considered to be a primary goitrogen and the increase in ^{131}I uptake is attributed to a secondary effect resulting from an increase in serum TSH.

In a 14-day biliary excretion study, pendimethalin caused decreases in serum T_3 and T_4 and an increase in TSH. There was increased liver weight, bile flow and cumulative biliary excretion of ^{125}I-T_4 glucuronide which was attributed to T_4-glucuronytransferase activity. There was also a decrease in T_4 binding to its specific transport protein (transthyretin) in the 56-day thyroid function study. The increased metabolic clearance of T_4 and enhanced biliary excretion of T_4 and T_4-glucuronide all contribute to the decrease in thyroid hormones.

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 and the 56-day thyroid function study.

e. Reversibility of lesions after exposure is terminated:

In the 56-day thyroid function study, pendimethalin was administered to male rats for 28 days, followed by a 28-day recovery period. After the end of 28 days, T_3 and T_4 were decreased, TSH increased and hypertrophy of follicular cells was observed. After the 28 day recovery period, hypertrophy of the follicular cells reversed, and T_3, T_4 and TSH returned to levels comparable to controls.

f. SAR to other thyroid tumorigens:

Pendimethalin is structurally related to trifluralin and oryzalin with reservations noted in the SAR section of the Cancer Peer Review document.

Based on the overall judgment of the six indicators in Factor I, it may be concluded that there is sufficient 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 considered by the Cancer Peer Review Committee were equivocal. There were some possible indications of mutagenic activity in the point mutation tests (frame shift). Although one host mediated assay was negative, a second test was questioned. A second Ames test, HGPRT (CHO), dominant lethal, in vitro cytogenetics (CHO) and DNA repair are negative.

In a microbial study submitted subsequently using Salmonella and E.coli produced negative effects when tested with the same batch of technical pendimethalin used in the 1991 carcinogenicity study that produced positive thyroid tumors. Two additional microbial tests were conducted using a purified sample of technical pendimethalin (99.5%) and a 1991 batch of technical pendimethalin. Negative results were obtained in the assays. A new CHO/HGPRT study was conducted that produced negative results. In addition, a new alkaline elution assay was conducted that produced negative results. The evidence submitted to date indicate that pendimethalin is not genotoxic to mammalian somatic and germ cells.

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

The evidence, when taken collectively, indicates that:

- pendimethalin is not genotoxic to mammalian cells and
- that the production of thyroid tumors (the only tumor type produced) may be attributed to **the disruption of the thyroid-pituitary hormonal balance.**

As a result of this determination, threshold considerations should be considered in the carcinogenic risk assessment of pendimethalin.

C. Developmental Toxicity

The rat developmental toxicity study is supplementary and, thus, does not satisfy the guideline requirement (83-3). However, this study in conjunction with the rabbit developmental toxicity study can be used to satisfy the guideline requirement (83-3). In order for the rabbit developmental toxicity study to be considered acceptable, individual litter data (fetal alterations) and historical control data must be submitted. However, if these data are not submitted, a confirmatory developmental toxicity study in a species to be determined, may have to be conducted.

Reviewed by: William B. Greear, M.P.H. William B. Greear 1/29/96 82-SS
Review Section IV, Toxicology Branch I (7509C) Rat
Secondary Reviewer: Marion P. Copley, D.V.M.
Review Section IV, Toxicology Branch I (7509C) Marion Copley 2/2/96

DATA EVALUATION REPORT

STUDY TYPE: 56-Day Thyroid Function Study-Rat
Guideline Series 82-SS

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-dinitrobenzenenamine

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 special 56-day feeding study to determine thyroid function (MRID 43135001), groups of 65-70 (5-15 per sacrifice time) male Crl:CD(SD) rats were treated at dose levels of 0, 500 or 5000 ppm (0, 31 or 292 mg/kg/day) of AC 92,553 (pendimethalin, 92.6%, Lot #AC5213-72A) 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%) and increased percent free T_3 (13%), 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. **The LOEL was 500 ppm (31 mg/kg/day based on thyroid effects. The NOEL could not be determined.**

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).

Table 1. Animal Assignment
56-Day Thyroid Function Study

		Day of Sacrifice						
Test Group	Dose in Diet (ppm)	-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+4°F, a relative humidity of 50±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).

Table 2: Body Weight (g)[1] and Body Weight Gain (g)[2].

	Day						
Dose Level (ppm)	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).

Table 3 Food Consumption[1]

	Days				
Dose Level (ppm)	0-8	8-15	22-29	36-43	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 $p < 0.05$

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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.

Table 3 - Absolute and Relative Thyroid Weights[1]

Organ	Control	500 ppm	5000 ppm
Day 28			
Thyroid (mg)	20.680	21.300	23.733*
% Bwt.	0.0043	0.0045	0.0053*
Day 56			
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).

6. 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 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 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	Control	500 ppm	5000 ppm
	Day 28		
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 (0^2x10^2)	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^2x10^2)	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.

EPA Reviewer: William.B. Greear,M.P.H William B. Greear 1/29/96
Review Section IV, Toxicology Branch I (7509C)
EPA Secondary: Marion P. Copley, D.V.M.
Review Section, Toxicology Branch I (7509C) Marion Copley 1/30/96

DATA EVALUATION REPORT

Study Type: 14-Day Intrathyroidal Metabolism Study
Guideline Series 85-1

EPA ID No.: MRID No.: 431350-03
Pesticide Chemical Code: 108501
Tox Chemical No.: 454BB
DP Barcode: D201875
Submission No.: S463228

Test Material: AC 92,533

Synonyms: Pendimethalin, Prowl, N-(-1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine

Sponsor: American Cyanamid Co.
Princeton, NJ 08543-0400

Testing Facility: Division of Endocrinology, University of Massachusetts Medical School , Worchester, MA 01655

Title of Report: A 14-Day Intrathyroidal Metabolism Study in Male Rats with AC 92,553

Study Number: UM-91-06-01

Authors: William J. DeVito and Lewis E. Braverman

Report Issued: April 16, 1993

Executive Summary: In a special 14-day feeding study to determine thyroid function (MRID 43135003), AC 92,553 (Pendimethalin, 92.6%, Lot #AC5213-72A) was administered in the diet to groups of 10 male Crl:CD(SD) rats at dose levels of 0, 100 or 5000 ppm (corresponding to 0, 10 or 500 mg/kg/day).

At 5000 ppm AC 92,533 for 14 days, TSH was increased and T_4 and T_3 were decreased. No treatment related effects were observed for rT_3 levels, thyroid weight, ^{131}I uptake in MIT, DIT or T_4. There was a significant increase of ^{131}I uptake by the thyroid of rats in the 5000 ppm group and an increase in incorporation of ^{131}I in T_3. Total T_3 and T_4 levels in the thyroid were not affected by treatment at 5000 ppm. The LOEL is 5,000 ppm (500 mg/kg/day) based on thyroid effects. The NOEL is 100 ppm (10 mg/kg/day).

The study is core-supplementary and does not by itself satisfy the guideline requirement for a series 85-1 metabolism study.

A. Materials

1. Test compound: AC 92,533; Description: orange solid; Lot No.: AC 6539-77A; Purity: 92.98%, Contaminants: not reported.

2. Test animals: Species: rat; Strain: Crl: CD(SD); Age: 13 weeks, Weight: not reported; Source: Charles River Laboratories, Inc.

B. Study Design:

14-DAY INTRATHYROIDAL METABOLISM STUDY

Test Group	Dose in Diet (ppm)	Number of Rats
Control	0	10
Low	100	10
High	5000	10

Rats were maintained under "controlled" conditions with a 12 hour on/off light cycle. Rats were maintained on their respective diets for 14 days after which blood samples were obtained by retroorbital bleeding and the serum was frozen at -20 degrees C and later analyzed for serum TSH, T_3, T_4 and rT_3. Each rat then received an ip injection of 25-50 μCi ^{131}I(NaI). Two hours after injection, the rats were sacrificed and blood was collected. One 50 μl aliquot was counted. A 10-20 μl aliquot was subjected to paper electrophoresis. The percentage of ^{131}I present in the organic forms was determined by counting the appropriate zones by autoradiography. An aliquot of homogenate from each thyroid was hydrolyzed with 20 mg of pancreatin at 37 degrees C. Then the homogenate was subjected to ascending paper chromatography in butanol-ethanol-0.5 N ammonia (5:1:2) solvent system. Monoiodotyrosine (MIT), diiodotyrosine(DIT), T3, and T4 were localized and counted. At termination, body weight and thyroid weight were determined.

C. Results

There was a significant increase in TSH (60%) in the 5000 ppm group when compared to the control and 100 ppm groups. In the 5000 ppm group there were significant decreases in T_4 (80%) and T_3 (39%). No treatment related effects were noted on rT_3 or

thyroid weight. There was an increase in ^{131}I uptake in rats in the 5000 ppm group (73%) when compared to controls (see Table 1). There were no significant increases of ^{131}I uptake in MIT, DIT or T_4. There was a significant percentage of ^{131}I incorporation into T_3 in rats fed 5000 ppm AC 92,553 (see Table 2). Ingestion of 5000 ppm did not significantly affect the total concentration of T_3 or T_4 in the thyroid.

Table 1. Thyroid Function at 14 Days

Parameter	Dose Level(ppm) 0	100	5000
TSH (μU/ml)	46.04 ±21.39	41.07 ±18.08	73.82 ±33.90
T_4 (μg/dl)	5.23 ±1.02	4.12 ±1.00	1.02 ±0.28
T_3 (ng/ml)	96.42 ±20.85	76.66 ±19.07	58.40 ±13.15
rT_3 (pg/ml)	70.92 ±17.83	63.01 ±16.72	48.52 ±9.76
^{131}I uptake % dose	2.75 ±1.04	2.85 ±1.06	4.75 ±0.63
Thyroid Wt. (mg)	27.85 ±7.66	26.67 ±3.67	26.27 ±4.24

D. Discussion

Oral exposure of rats to 5000 ppm AC 92,533 increased TSH, and decreased T_4 and T_3 levels. No treatment related effects were observed for rT_3 levels, thyroid weight, ^{131}I uptake in MIT, DIT or T_4. There was a significant increase in incorporation of ^{131}I in T_3. Total T_3 and T_4 levels in the thyroid were not affected by treatment at 500 ppm. No treatment related effects were noted in the 100 ppm group.

The study is core-supplementary. It does not by itself satisfy the guideline requirement for a series 85-1 metabolism study.

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Pages ____ through ____ are not included.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

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EPA Reviewer: William B. Greear, M.P.H. William B. Greear 1/29/96 82-SS
Review Section IV, Toxicology Branch I (7509C) Rat
EPA Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 3/3/96
Review Section IV, Toxicology Branch I (7509C)

DATA EVALUATION REPORT

Study Type: Biliary Excretion and Hepatic Metabolism
Guideline Series 82-SS

EPA ID Nos.: MRID NO.: 431350-04
Pesticide Chemical Code: 108501
Tox Chemical No.: 454BB
DP Barcode: D201875
Submission No.: S463228

Test Material: AC 92,553

Synonyms: Pendimethalin, Prowl, N-(1-ethypropyl)-3,4-dimethyl-2,6-dinitrobenzeneamine

Sponsor: American Cyanamid, Princeton, NJ 08543-0400

Testing Facility: Division of Endocrinology, University of Massachusetts Medical School, Worcester, MA 01655

Title of Report: Effect of Ingestion of AC 32,553 on Biliary Excretion and Hepatic Metabolism

Study Number: UM-92-03-01

Authors: William J. DeVito, Lewis E. Braverman

Report Issued: April 16, 1993

Executive Summary: In a second special 14-day feeding study to determine biliary excretion and hepatic metabolism, AC 92,553 (pendimethalin, 92.98%, Lot #AC6539-77A) was administered to groups of 10 male Crl:CD(SD) rats at dose levels of 0, 100 or 5,000 ppm (corresponding to 0, 10 or 500 mg/kg/day).

Ingestion of 5000 ppm produced decreases in serum T_3 and T_4 with a compensatory increase in TSH. Also increased were liver weight, bile flow and cumulative biliary excretion of ^{125}I-T_4 with a slight increase in T_4-glucuronytransferase activity detected by generation of ^{125}I-T_4 glucuronide from ^{125}I-T_4 in vitro by hepatic microsomes. The increase in enzyme activity was also demonstrated in vivo by a significant increase in biliary excretion of ^{125}I-T4-glucuronide. The LOEL is 5,000 ppm (500 mg/kg/day) based on thyroid effects. The NOEL is 100 ppm (10 mg/kg/day).

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

A. Materials

1. Test compound: AC 92,553; Description: orange solid; Lot No.: AC 6539-77A; Purity: 92.98%; Contaminants: not reported.

2. Test animals:Species: rat; Strain: Crl: CD(SD); Age 13 weeks;Weight: not reported; Source: Charles River Laboratories, Inc.

B. Study Design

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

Table 1. 14-Day Biliary Excretion of Hepatic Metabolism Study in Male Rats

Test Group Group	Dose in Diets (ppm)	Main Study 14 Days
Control	0	10
Low	100	10
High	5000	10

The animals received the test diets for 14 days. On day 14 each rat was anesthetized with ketamine and a laparotomy was performed to expose the bile duct. The bile duct was cannulated with polyethylene tubing. Each rat was then injected via the tail vein with 25-30 µi [^{125}I]-T_4. Bile was collected at one-half hour intervals for the first two hours and at one hour intervals for the last two hours.

Each fraction of bile was counted for radioactivity by pooling half of the bile volumes collected from each rat at each hour interval. Samples of pooled bile was spotted on a silica gel TLC plate to separate T_4-glucuronide, T_4 and iodide. The fraction of total radioactivity as T_4-glucuronide was calculated and the total $^{125}T_4$-glucuronide excreted was calculated as a percentage of the administered dose. After collection of the bile, the livers were extracted, weighed, homogenized in water and the total radioactivity calculated and presented as a percentage of the administered dose per gram of liver. T_4-glucuronyltransferase activity in liver microsomes was determined by TLC analysis and expressed as picomoles/mg protein/min. Blood samples were taken after 14 days and were analyzed for TSH, T_3 and T_4 after light anesthesia.

C. Results

As can be seen in Table 2, there was a significant increase in serum TSH levels in rats fed 5000 ppm as well as decreases in serum T_4 and T_3 levels. An increase in liver weights of almost 40% in animals in the 5000 ppm group was observed. There was no increase in hepatic uptake of ^{125}I-T_4 when expressed as a percentage of the administered dose per gram of liver. There was a significant increase in bile

flow in rats in the 5000 ppm group. There was also significant increases in biliary excretion of ^{125}I-T_4 and ^{125}I-T_4-glucuronide, generated by glucuronyltransferase in hepatic microsomes, in rats in the 5000 ppm group. Glucuronyltransferase activity was only slightly (not significant) increased in the 5000 ppm group.

Table 2. Select Values in Male Rats Following 14 Days of AC 92,553 Intake[1]

Dose	Control	100 ppm	5000 ppm
n=	6	7	6
TSH (μU/ml)	54.76 ±20.62	60.7 ±28.75	103.8* ±41.81
T_3 (μg/dl)	87.94 ±18.32	79.74 ±18.53	56.7* ±15.67
T_4 (ng/dl)	5.81 ±0.17	4.82 ±0.82	2.0* ±0.76
% Hepatic uptake of ^{125}I-T_4/liver	24.96 ±3.45	23.92 ±3.09	28.09 ±4.17
% Hepatic uptake of ^{125}I-T_4/g liver	1.43 ±0.19	1.57 ±0.24	1.38 ±0.21
Bile flow (ml/4 hrs)	6.77 ±0.99	6.01 ±1.14	8.12* ±1.23
% Bil. Excr. of ^{125}I-T_4	6.44 ±1.22	8.75 ±4.60	9.95* ±2.68
% Bil. Excr. of ^{125}I-T_4-glucuronide	0.144 ±0.025	0.141 ±0.040	0.234* ±0.071
Gluc. transferase act. (pmol/min/mg)	2.73 ±0.36	2.44 ±0.50	3.21 ±1.29

* $p < 0.05$ vs. control and low-dose group
[1]-data extracted from pp. 26-29,31.

D. Discussion

Ingestion of 5000 ppm AC 92,533 in the diet for 14 days produced decreases in serum T_3 and T_4 with a compensatory

increase in TSH_4 increases in liver weight, bile flow and cumulative biliary excretion of ^{125}I-T_4 and ^{125}I-T_4-glucuronide with a slight increase in T_4-glucuronyltransferase activity detected by generation of ^{125}I-T4-glucuronide from ^{125}I-T_4 *in vitro* by hepatic microsomes. The increase in enzyme activity was also demonstrated *in vivo* by a significant increase in biliary excretion of ^{125}I-T_4-glucuronide by rats treated with 5000 ppm AC 92,553.

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

Primary Review by: Roger Gardner Roger Gardner 1/2/96
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Pamela Hurley Pamela M. Hurley 1/2/96
Review Section 1, Toxicology Branch I/HED

DATA EVALUATION RECORD

This DER supercedes HED document number 002406.

Study Type: Developmental Toxicity
Guideline §83-3(b)
Species: Rabbit

EPA Identification No.s: EPA MRID No. 00117444
EPA Pesticide Chemical Code: 108501
Submission No. none
Data Package No. none

Test Material: AC 92,553 (Pendimethalin)

Synonyms: N-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine

Sponsor: American Cyanamid

Study Number(s): 362-163

Testing Facility: Hazleton Laboratories America, Inc., Vienna, VA

Title of Report: Teratology Study in Rabbits: AC 92,553 Final Report.

Author(s): Rice, Anna E.

Report Issued: May 11, 1981

Executive Summary: Pendimethalin was administered in corn oil to groups of 20 artificially inseminated New Zealand White strain rabbits by gavage at dose levels of 0, 15, 30, or 60 mg/kg/day from gestation day 6 through 18 (MRID 00117444). Females were observed for signs of toxicity, and body weights were measured during gestation. Animals were sacrificed on gestation day 29 and reproductive observations were made and uteri were examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

No maternal toxicity was reported at doses ≤60 mg/kg/day (highest dose tested). However, the range-finding study indicated that doses ≥ 125 mg/kg/day were associated with increased mortality (3/5, 5/5 and 4/5 in the 125, 250, and 500 mg/kg/day, respectively compared with 0/5 in the control group).

A slight increase in the mean incidence of skeletal anomalies in the mid- and high-dose groups which consisted of findings of less than twelve pairs of ribs (0/111, 1/118 and 4/107 fetuses in the control, mid-, and high-dose groups, respectively, not statistically significant) and/or missing or incompletely ossified vertebrae (0/111, 1/118 and 7/107 fetuses in the control, mid and high dose groups, respectively). No individual litter data or historical control data were available in the report to support a conclusion regarding the significance of these alterations. A developmental toxicity NOEL could not be determined from this study.

Core Classification: This study does not satisfy §83-3 guideline requirements for a rabbit developmental toxicity study and should be classified as Supplementary. It is upgradable pending receipt

of individual litter data (fetal alterations) and historical control data.

Materials and Methods

A. Test Animals: Mature virgin female New Zealand White strain rabbits were used. They were acclimated for 22 days at the laboratory. The animals were from Dutchland Laboratory Animals, Inc. Denver, PA.

B. Mating Procedures: The mating procedure was described in the report as follows:

> Male New Zealand White rabbits, maintained in this laboratory as breeding stock, served as sperm donors for the artificial insemination.
>
> Ovulation was induced in each female by intravenous injection of 250 IU of human chorionic gonadotropin. Approximately six hours after injection females were artificially inseminated...The day of insemination was designated as Day 0 of gestation.

C. Test Substance: Technical grade AC 92,553 (pendimethalin; 92.2% a.i.) was supplied as a solid (lot no. 3525-129-1), and the dosages were adjusted to 100% active ingredient according to the report. The report also stated, "Information on the stability and methods of synthesis, as well as data on composition or other characteristics which define the test material, are on file with the sponsor."

D. Vehicle: Corn oil.

E. Dose Solution and Preparation: Doses were selected on the basis of results from a range-finding study discussed in the "Appendix" of this DER.

The test substance was suspended in the vehicle and was administered in 1.0 ml/kg. Dose suspensions were based on gestation day 6 body weights according to the report. Samples of each dosing solution were sent to the sponsor for analysis to verify the test substance's concentration.

F. Study Design: Mated animals were assigned to four groups as follows:

Test Group	Dose Level (mg/kg/day)*	Number Assigned
Control	0	20
Low dose	15	20
Mid dose	30	20
High dose	60	20

* Doses were administered by gavage on gestation days 6 through 18.

G. Observations: The animals were observed daily for mortality, changes in appearance and behavior, and clinical signs. They were weighed on gestation days 0, 6, 9, 12, 15, 18, 24 and 29 of gestation.

On gestation day 29 surviving animals were sacrificed and examined for gross pathological findings.

The ovaries and uterus were removed, weighed, and examined to determine the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses.

The report described fetal examinations as follows:

> Visceral Examination of Fetuses
> All of the fetuses were opened by longitudinal incision, the sex determined, and examined grossly both externally and internally. The heads of approximately one-third of the fetuses were removed, fixed in Bouin's solution, sectioned by Wilson's freehand razor technique for examination of the eyes, palate, nasal septum, and brain, and sealed in plastic. The prepared sections were then re-examined against a light box with the aid of magnification.
>
> Skeletal Examination of Fetuses
> Following visceral examination, all fetuses were eviscerated and placed in 95% ethyl alcohol. After proper fixation and dehydration, skeletons were stained with alizarin red solution. Each skeleton was examined for anomalies, degree of ossification, and bone alignment with the aid of magnification on a light box.

H. Statistical Analysis: Statistical analyses were described in the report as follows:

> The maternal body weight change(s), uterine weight (gravid and nongravid), and mean fetal weight and length data of the

control group were compared statistically to data of the treated groups of the same sex by Bartlett's test for homogeneity of variance and the one-way classification analysis of variance (ANOVA). If significant results were obtained from both Bartlett's test and ANOVA, a multiple pairwise comparison procedure was used to compare the group mean values. If a significant result was not obtained from Bartlett's test, but was obtained from ANOVA, Scheffe's multiple pairwise comparison procedure was used to compare the group mean values. The fetal skeletal anomaly data was statistically compared using Fisher's exact test for comparing two proportions. The percent of male fetuses, reproduction and viability indices, and the percentage of visceral and skeletal variances were analyzed by nonparametric ANOVA. The litter was used as the experimental unit. All analyses were evaluated at the 5.0% probability (one-tailed) level.

I. Historical Control Data: Historical control data were not provided with this report.

Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report described these results as follows:

One control animal, three mid-dose animals, and one high-dose animal were found dead or sacrificed in extremis during the study.

A higher incidence of anorexia and adipsia was noted in the high-dose animals during the treatment and post-treatment phases.

These results are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	15	30	60
No. with anorexia/no. examined during days				
0-5	--	--	--	--
6-18	4/20	4/20	6/20	8/20
19-29	5/20	5/20	7/17	7/19
No. with adipsia/no. examined during days				
0-5	--	--	--	--
6-18	2/20	3/20	2/20	6/20
19-29	3/20	3/20	3/17	6/19

2. Body Weight and Food Consumption: The report described the body weight results as follows:

> Statistical evaluation of the body weight change data revealed a significantly lower mean change for Days 0-6 when the mid-dose animals were compared to the control animals. This finding can not be considered related to treatment since treatment was not initiated until Day 6. No treatment-related trends were noted in mean body weights or body weight changes.

These results are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	15	30	60
Mean body weight (g) on gestation day				
0	3345.5	3910.8	3327.3	3458.2
6	3458.4	3503.3	3358.5	3525.0
9	3511.1	3549.2	3424.8	3550.0
12	3530.5	3618.8	3456.9	3542.4
15	3553.7	3648.9	3525.0	3623.3
18	3562.9	3663.3	3522.9	3578.6
24	3630.3	3746.9	3635.9	3685.6
29*	3715.0	3817.5	3694.4	3794.4
Mean body weight gain (g) gestation days				
0 - 6	112.9	92.5	31.3*	66.8
6 - 18	104.5	160.0	123.2	57.8
18 - 29	135.6	154.2	171.5	215.8
0 - 29	361.7	406.7	388.2	340.6

* Significantly different from controls, $p \leq 0.05$.

3. Gross Necropsy and Uterine Observations: The report noted that no significant differences in group mean uterine weights were observed in the study.

Gross necropsy observations were described in the report as follows:

> Compound-like material was noted in the chest/thoracic cavity, thymus, diaphragm, and/or adipose tissue of the mid- and high-dose animals which were sacrificed at termination. Abnormal liver findings were noted only in the mid- and high-dose animals which were found dead or sacrificed in extremis, although findings like these are observed at necropsy in New Zealand White rabbits sacrificed at (this laboratory).

There were only one or two animals in each group with the above mentioned observations.

No significant effects were noted on intrauterine observations. These results are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	15	30	60
Number of animals	20	20	20	20
Number died:	1	0	3	1
Non-pregnant	1	2	0	1
With viable fetuses at termination	17	18	17	18
Corpora lutea/doe	12.3	10.3	14.4	10.3
Implantations/doe	7.4	6.8	8.1	6.9
Resorptions/litter	1.3	0.9	1.2	1.0
Mean litter size	6.2	5.8	6.1	5.9
Mean gravid uterus weight (g)	371.82	366.10	412.07	375.11
Mean fetal weight (g)				
males	41.03	43.06	41.44	42.5
females	41.39	42.00	40.94	42.7
Mean fetal length (cm)				
males	9.24	9.31	9.19	9.3
females	9.32	9.18	9.19	9.3
% males	42.5	52.4	47.8	57.4

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

The report described the incidence of fetal effects as follows:

> Statistical evaluation of the skeletal anomalies and the percent of visceral anomalies and variants did not reveal any significant differences. However, there was an increase in the mean incidence of skeletal anomalies (less than 12 pairs of ribs and/or missing/incomplete vertebral column) with a decrease in the mean incidence of skeletal variants in the mid- and high-dose groups compared to control. In addition, there was an increase in the skeletal variants in the control group which consisted primarily of the findings of incomplete closure of the skull, incomplete/nonfused thoracic centra, and fused or malaligned sternebra.

The results for skeletal observations are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	15	30	60
No. of fetuses examined	111	106	118	107
No. of live fetuses	111	105	117	107
No. of normal fetuses	76	78	93	82
No. dead or resorbed	0	1	1	0
No. with anomalies	0	0	2	5
No. with variants	35	27	23	22
No. of anomalies	0	0	2	11
<12 pairs of ribs	0	0	1	4
Vertebrae missing/incomplete				
Lumbar	0	0	1	2
Sacral (incomplete)	0	0	0	2
Caudal	0	0	0	3
Number of litters with anomalies	0	0	2	5
Number of litters with variants	35	27	23	22
Incidence of anomalies (%)*	0.0	0.0	3.8	3.6
Incidence of variations (%)*	38.4	25.4	19.8	20.5

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.
* The report noted, "Mean values calculated on a per litter basis."

Discussion

A. Authors' Conclusions: The authors' conclusion was reported as follows:

> Maternal survival was comparable between the control and treated groups...Anorexia and adipsia were noted more frequently in the high-dose animals than in controls during the treatment and post-treatment phases. Mean body weights and weight changes were comparable between the control and treated groups. At necropsy, compound-like material was found in the chest/thoracic cavity, thymus, diaphragm and/or adipose tissue. in most mid- and high-dose animals.
>
> Pregnancy rates, the numbers of corpora lutea and the mean implantation efficiency were comparable between the control and treated groups. Although not statistically significant, a lower incidence of resorptions and higher incidence of fetal viability were observed in the high dose group when compared to controls.
>
> The mean percent of males per litter exhibited normal biological variation. Mean fetal weights and lengths were comparable among all groups.

There were no significant differences in the incidences of visceral anomalies or variants, or skeletal anomalies or variants between the treated and control groups. There was a slight increase in the mean incidence of skeletal anomalies in the mid- and high-dose groups which consisted of findings of less than twelve pairs of ribs and/or missing vertebral column unique to fetuses in the mid- and high-dose groups. There was also a slight increase in skeletal variants in the control group in which the finding observed most frequently was incomplete/nonfused thoracic centra.

B. Reviewer's Discussion and Conclusions: Although the authors noted anorexia during the treatment and post-treatment phases of the study (see page 4 above), there were no significant effects on body weights and body weight gains (see page 5 above). It should be noted that there were 4/20 animals reported to exhibit anorexia in the control group of the main study, and similar results were noted in the control and treated groups of the range finding study (see page 12 in the Appendix below). The incidence of anorexia in the control group during the post treatment period (5/20) was also not significantly less than that in the mid- and high-dose groups (7/20 in both groups). Therefore, it is unlikely that the reported clinical observations of anorexia in the study are toxicologically significant.

The report noted that the litter was considered the experimental unit for determining significant developmental effects (see page 4 above). However, the number of litters containing affected fetuses was not reported, and no individual litter data on the incidence of fetuses with visceral and skeletal anomalies or variations were included in the report. Since the investigators noted slight increases in certain anomalies (see page 7 above), these data would be important to an interpretation regarding the significance of the slight increases in the incidence of anomalies noted by the authors.

Further, the percentage incidence expressed in the report (see table on page 7 above) is a proportion based on affected fetuses per total fetuses in a litter. As would be expected, the value reported should be somewhat different from the proportion calculated on a total affected fetuses/total fetuses examined basis. For example, [11 ÷ 107] x 100 = 2.8% which is less than the 3.6% reported for skeletal anomalies. If the average litter size of a litter in the high-dose group is 5.9, then one affected fetuses results in a litter's value of [1 ÷ 5.9] x 100 = 16.9%. There were 18 litters in the high dose group, and if five of those litters had a 16.9% incidence, the groups mean percent incidence on a litter basis would be [16.9% x 5] ÷ 18 = 4.6%/litter. Therefore, it

is reasonable to assume that more than one litter contains one or more fetuses with skeletal anomalies to result in the 3.6% incidence/litter reported for the high-dose group.

In any event, the reported incidences are low, and the control group had no incidences of skeletal anomalies, so that historical control data would also be necessary to support a final interpretation of the noted increased incidences of fetal effects.

The results of the range-finding study, particularly the incidence of mortality at levels ≥125 mg/kg/day (see page 12 in the Appendix below), suggest that the choice of doses for the main study should be appropriate despite the apparent lack of maternal toxicity at the doses tested in the main study (15, 30, and 60 mg/kg/day).

Title of Report: Pilot Teratology Study in Rabbits: AC 92,553 Revised Final Report.

Author(s): Rice, Anna E.

Report Issued: May 6, 1981

Executive Summary: Pendimethalin was administered in corn oil to groups of 5 artificially inseminated New Zealand White strain rabbits by gavage at dose levels of 0, 31.25, 62.5, 125, 250 or 500 mg/kg/day from gestation day 6 through 18 (MRID 00117444). Females were observed for changes in appearance or behavior, and body weight during gestation. Animals were sacrificed on gestation day 29 and reproductive observations were made and uteri were examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external and visceral alterations.

Maternal toxicity was reported at doses ≥ 125 mg/kg/day and was indicated by increased mortality (3/5, 5/5 and 4/5 in the 125, 250, and 500 mg/kg/day dose groups, respectively compared with 0/5 in the control group).

Increased resorptions was the only developmental effect attributed to administration of pendimethalin at 62.5 mg/kg/day, but litter sizes were comparable at that level and in the control group. The increased mortality at doses of 125 mg/kg/day or greater resulted in too few litters for analysis of possible effects on resorptions. Therefore, the increased resorptions are not toxicologically significant.

These results suggest that dose levels as high as 62.5 mg/kg/day administered by oral gavage may be adequate for a full developmental toxicity study.

Core Classification: This study does not satisfy §83-3 guideline requirements for a rabbit developmental toxicity study and should be classified as Supplementary. It is a pilot study conducted for the purpose of establishing appropriate dose levels for a full developmental toxicity study.

Materials and Methods

A. Test Animals: Adult virgin female New Zealand White strain rabbits were used. They were described as young adults on arrival at the laboratory and were acclimated for six weeks. Animals selected for the study weighed 3090 - 5500 g. The animals were from Dutchland Laboratory Animals, Inc. Denver, PA.

B. Mating Procedures: The mating procedure was described in the report as follows:

Male New Zealand White rabbits, maintained in this laboratory as breeding stock, served as sperm donors for the artificial insemination.

C. Test Substance: Technical grade AC 92,553 (pendimethalin; 92.2% a.i.) was supplied as a solid (lot no. 3525-129-1), and the dosages were adjusted to 100% active ingredient according to the report. The report also stated, "Information on the stability and methods of synthesis, as well as data on composition or other characteristics which define the test material, are on file with the sponsor."

D. Vehicle: Corn oil.

E. Dose Solution: The test substance was suspended in the vehicle and was administered in an unspecified volume. Dose suspensions were based on gestation day 6 body weights according to the report. The report did not indicate whether samples of each dosing solution were analyzed to verify the test substance's concentration or stability.

F. Study Design: Mated animals were assigned to four groups as follows:

Test Group	Dose Level (mg/kg/day)*	Number Assigned
1	0	5
2	31.25	5
3	62.5	5
4	125	5
5	250	5
6	500	5

* Doses were administered by gavage on gestation days 6 through 18.

G. Observations: The animals were observed twice daily for mortality and once daily for "pharmacological and toxicological signs." They were weighed on gestation days 0, 6, 12, 18, 24 and 29 during the study. Food consumption was grossly evaluated by noting excretion on a daily basis during gestation.

On gestation day 29 surviving animals were sacrificed and examined for gross pathological findings.

The ovaries and uterus were examined to determine the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses.

The report stated, "Fetuses were...individually identified, examined grossly, and weighed."

H. Statistical Analysis: No statistical analyses were described in the report.

I. Historical Control Data: Historical control data were not provided with this report.

Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report described these results as follows:

> No animals were found dead prior to day 29 in Group 1; however, one animal was found dead prior to Day 29 in Group 2, no animals in Group 3, three animals in Group 4, five animals in Group 5, and four animals in Group 6. In addition, one animal in Group 2, one in Group 3, and two in Group 4 were sacrificed following signs of abortion (placenta-like substance or blood found in the trays).
>
> Clinical observations noted only in the treated animals during or after the dosing period included depression (noted most frequently), coldness, cyanosis, paleness, and soft feces or compound-colored feces. Anorexia was observed in most control and treated animals during and following the dosing period.

2. Body Weight and Food Consumption: The report noted:

> Body weights were lower in treated Groups 3 through 6; however, a more specific comparison of group values was not possible due to the high mortality in Groups 4 through 6.
>
> Incidences of inappetence were noted in both the control and treated groups during the study without any compound-related frequency evident.

Therefore, results are not summarized from tables in the report.

3. Gross Necropsy and Uterine Observations: The report described gross necropsy observations as follows:

> Overall, necropsy findings were noted most frequently in Groups 3 through 6 and more specifically in those animals which were found dead or sacrificed prior to Day 29. The liver, kidneys,

stomach, pancreas, intestines, mesenteric lymph nodes, urinary bladder, mesentery and adipose fat were all noted to have a yellowish tinge in the treated groups. A higher incidence of thin/vascular stomach walls was also noted in Groups 4 through 6. Other necropsy findings were generally unremarkable and considered incidental.

Because of excessive mortalities in Groups 4 through 6, uterine observations are summarized from the first 3 treated groups as follows:

Observation	Dose level (mg/kg/day) 0	31.25	62.5
Number of animals	5	5	5
Number pregnant	5	4	4
Number died	0	1	0
Number surviving to Day 29	5	3	4
Corpora lutea/doe	12.8	10.8	15.0
Implantations/doe	5.6	4.3	6.5
Resorptions/litter	0.4	0.5	1.7
Dead fetuses/litter	0.2	0	0
Mean litter size	5.0	4.5	5.0
Mean fetal body weight (g) - males	42.43	51.55	39.37
- females	38.3	44.00	40.23
% males	51.8	53.5	64.3

B. Developmental End Points: Fetal observations were described in the report as follows:

Gross visceral examination of fetuses taken by Cesarean section on Day 29 of gestation revealed pale kidneys and heart and cloudy fluid in the abdominal cavity of a Group 1 fetus (which was dead on delivery). All fetuses from one Group 3 dam were described as small.

Examination of fetuses from dams not surviving to Day 29 revealed the following: five fetuses with nonfused eyelids were noted from a Group 3 female sacrificed on Day 25 of gestation. One of these had intestines protruding from the umbilicus and two had slight dilation of the lateral ventricles, possibly due to immaturity. Two Group 4 females were sacrificed on Day 26. Observations in one litter consisted of three fetuses with nonfused eyelids; and one fetus with some dilation of the ventricles, dark material present in the skull, and some disorganization of the brain tissue. One fetus in the other litter was observed as having an arched pulmonary artery, transposed great vessels, and the aorta appeared to split into the right carotid and the right brachial arteries.

Discussion

A. Authors' Conclusions: The authors' conclusion was reported as follows:

> ...oral administration of AC 92,553...to pregnant rabbits from Days 6-18 of gestation produced maternal toxicity at levels of 125, 250, and 500 mg/kg/day as well as possible maternal toxicity.

B. Reviewer's Discussion and Conclusions: See "Executive Summary" above for discussion.

Primary Review by: Roger Gardner Roger Gardner 1/2/96
Review Section 1, Toxicology Branch 1/HED
Secondary Review by: Pamela Hurley Pamela M. Hurley 1/2/96
Review Section 1, Toxicology Branch I/HED

DATA EVALUATION RECORD

This DER supercedes HED Document numbers 00544, 007751, and 008558.

Study Type: Developmental Toxicity
Guideline §83-3(a)
Species: Rat

EPA Identification No.s: EPA MRID No. 00025752
EPA Pesticide Chemical Code: 108501
Submission No. none
Data Package No. none

Test Material: AC 92,553 (Pendimethalin, 94.2% a.i., lot no. AC 1984-79-3)

Synonyms: N-(1-Ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine

Sponsor: American Cyanamid

Study Number(s): 362-155

Testing Facility: Hazleton Laboratories America, Inc., Vienna, VA

Title of Report: Oral Teratology Study in Rats: AC 92,553 Final Report.

Author(s): Mistretta, L.H., and P. Miller

Report Issued: August 17, 1979

Executive Summary: Pendimethalin (94.2% a.i.) was administered in corn oil to groups of 30 mated Sprague-Dawley CD strain rats by gavage at daily dose levels of 0, 125, 250, or 500 mg/kg/day from gestation day 6 through 15 (MRID 00025752). Females were observed for signs of toxicity, and body weights were measured during gestation. Animals were sacrificed on gestation day 21 and reproductive observations were made and uteri were examined for live fetuses and intra-uterine deaths. Fetuses were weighed, sexed, and examined for external, visceral and skeletal alterations.

There were no maternal or developmental effects noted at any dose level tested, and based on these results, the NOELs for developmental and maternal toxicity are ≥500 mg/kg/day (highest dose tested).

Core Classification: This study should be classified as Supplementary and used in conjunction with the rabbit developmental toxicity study (MRID 00117444) to satisfy guideline requirement §83-3. It is not upgradable because an adequate dose range may not have been tested.

Materials and Methods

A. Test Animals: Female Sprague-Dawley CD strain rats weighing from 180 to 200 g. were used. They were acclimated for two weeks at the laboratory. The animals were from A.R.S. Sprague Dawley, Madison, WI.

B. Mating Procedures: The mating procedure was described in the report as follows:

> ...the rats were mated by placing one male with two females in a breeding cage until a sufficient number of females were impregnated (no longer than three weeks). A vaginal examination of each female was performed daily with a microscopic examination of a slide prepared with a small drop of normal saline delivered into and then recovered from the vagina with a pipette. The slide was examined for the presence and viability of sperm. The day of observation of sperm or vaginal plug was designated as Day 0 of gestation, and the females were then assigned consecutively to an experimental groups...

C. Test Substance: Technical grade AC 92,553 (pendimethalin; 94.2% a.i.) was supplied as a solid (lot no. 1984-79-3), and the dosages were adjusted to 100% active ingredient according to the report.

D. Vehicle: Corn oil.

E. Dose Solution and Preparation: The test substance was suspended in the vehicle and was administered in an unspecified volume to each animal. Dose suspensions were based on gestation day 6 body weights according to the report. Samples of each dosing solution were sent to the sponsor for analysis to verify the test substance's concentration.

F. Study Design: Mated animals were assigned to four groups as follows:

Test Group	Dose Level (mg/kg/day)*	Number Assigned
Control	0	33
Low dose	125	34
Mid dose	250	33
High dose	500	33

* Doses were administered by gavage on gestation days 6 through 15.

G. Observations: The animals were observed daily for mortality, changes in appearance and behavior, and clinical signs. They were weighed on gestation days 0, 6, 9, 12, 15, and 20 of gestation and food consumption was determined on days 6, 9, 12, 15, and 20.

On gestation day 20 surviving animals were sacrificed and examined for gross pathological findings.

The ovaries and uterus were removed, weighed, and examined to determine the numbers of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses.

The report described fetal examinations as follows:

> Visceral Examination of Fetuses
> One third of the fetuses were fixed in Bouin's solution, sectioned by Wilson's freehand razor technique, and sealed in plastic. Whole body transverse sections of the head, thoracic and abdominal regions were taken and examined for visceral abnormalities with the aid of magnification.
>
> Skeletal Examination of Fetuses
> The remaining two-thirds of the fetuses from each litter were examined internally, eviscerated and placed in 95% ethyl alcohol. After proper fixation and dehydration, the skeletons were stained in potassium hydroxide-alizarin red solution and finally cleared in a solution of glycerol-ethyl alcohol and benzyl alcohol, and stored in glycerol-ethanol (50/50). Each skeleton was examined with the aid of magnification on a light box for ossification, bone alignment, and anomalies.

H. Statistical Analysis: Statistical analyses were described in the report as follows:

> The maternal body weight gains and food consumption data of the control groups were compared statistically to treated groups by Bartlett's test for homogeneity of variance and the one-way classification analysis of variance (ANOVA). If significant results were obtained from both Bartlett's test and ANOVA, a multiple pairwise comparison procedure was used to compare the group mean values. If a significant result was not obtained from Bartlett's test, but was obtained from ANOVA, Scheffe's multiple pairwise comparison procedure was used to compare the group mean values. The reproduction and viability indices were analyzed by either the chi-square method or by Wilcoxon's nonparametric comparison of group mean(s). All analyses were evaluated at the 5.0% probability level.

I. Historical Control Data: Historical control data were not provided with this report.

Reported Results

A. Maternal Observations:

1. Clinical Signs and Mortality: The report described these results as follows:

> Cloudy eyes, sores on the tail, and scales on the tail were the most frequently reported clinical observations. These observations occurred with a comparable frequency in all groups and are considered incidental. Urine stains occurred more frequently in the mid- and high dose animals on Days 6-15 than in the low-dose and control animals.

These results are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	15	30	60
No. with cloudy eyes / no. examined on days				
0 - 6	17/33	15/34	14/32	14/33
6 - 15	20/33	14/34	16/32	13/33
15 - 20	19/33	14/34	13/32	14/33
No. with sores on tail/ no. examined on days				
0 - 6	7/33	11/34	13/32	15/33
6 - 15	8/33	7/34	10/32	5/33
15 - 20	7/33	6/34	5/32	2/33
No. w. scales on tail/ no. examined on days				
0 - 6	2/33	9/34	2/32	4/33
6 - 15	2/33	4/34	4/32	3/33
15 - 20	3/33	3/34	2/32	1/33
No. with urine stains / no. examined on days				
0 - 6	0/33	0/34	0/32	0/33
6 - 15	0/33	0/34	4/32	14/33
15 - 20	0/33	0/34	5/32	*/33

* Number not readable in report reproduced for review.

2. Body Weight and Food Consumption: The report described the body weight results as follows:

> Analyses of mean maternal weight changes revealed no statistically significant differences between the control and

test groups calculated for Days 0-6, 6-15, 15-20, 0-20 of gestation.

Maternal food consumption was analyzed for Days 6, 9, 12, 15, and 20. Analyses revealed a statistically significantly higher mean value for the high-dose group when compared to the control group on Day 20 of gestation. No other statistically significant differences between the treated and control groups were noted at any of the other days analyzed.

3. Gross Necropsy and Uterine Observations; Gross necropsy observations were described in the report as follows:

At sacrifice (Day 20), the uterus was dissected with clear fluid in two control rats, two low-dose rats, and one mid-dose rat. All five of these females were found not pregnant on Day 20 of gestation. One low-dose rat had a tissue mass on its liver, and one high-dose rat had a nodule in the inguinal area. Two low-dose rats, three mid-dose rats, and eight high-dose rats had a yellow tinge to their body fat. Besides the greater incidence of yellow body fat in the high-dose group, no treatment-related tendencies were noted.

No significant effects were noted on intrauterine observations. These results are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	125	250	500
Number of animals	33	34	32	33
Number died:	0	0	0	0
Non-pregnant	4	5	4	3
With viable fetuses at termination	29	29	28	30
Corpora lutea/doe	13.76	14.0	14.04	15.13*
Implantations/doe	12.24	12.86	12.28	12.83
Resorptions/litter	0.62	0.69	0.39	0.6
Mean litter size	11.52	12.17	11.89	12.23
Mean fetal weight (g)	4.00	4.08	4.02	4.01
Mean fetal length (cm)	3.93	3.96	3.95	4.02

* No other statistically significant differences from controls were noted in the original report, $p \leq 0.05$.

The report described the incidence of fetal effects as follows:

The visceral examination revealed hydronephrosis in one mid-dose fetus. No other visceral anomalies were noted. Incidental findings of slight dilation of the kidneys and

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ureters were also noted; however, these are considered to be common variants in rats seen at Hazleton Laboratories.

The skeletal examination revealed anomalies of the ribs and vertebral column in one high-dose fetus. No other skeletal anomalies were noted. Incidental findings of lagging ossification of the skull, rib cage, vertebral column, pelvic girdle, and extremities; and angulated ribs were also observed. These findings are considered to be common variants seen in rats at Hazleton Laboratories.

The results for apparently dose-related skeletal observations are summarized from the report as follows:

Observation	Dose level (mg/kg/day) 0	125	250	500
No. of live fetuses examined	234	242	232	254
No. of normal fetuses	225	239	219	239
No. with lagging ossif. in extremities - fetuses*	8	6	11	13
- litters*	4	5	8	9

No statistically significant differences from controls were noted in the original report, $p \leq 0.05$.
* Data were obtained from individual animal data sheets.

Discussion

A. Authors' Conclusions: The authors' conclusion was reported as follows:

The maternal mean body weight, pregnancy rate, mean number of implantations, implantation efficiency, incidence of resorption, mean number of dead and live fetuses, incidence of fetal death, fetal viability and the mean fetal length and weight were statistically similar when the treated groups were compared to the control group. A significantly higher mean maternal food consumption value was noted on Day 20 when the high-dose group was compared to the control group, and there was a significantly higher mean number of corpora lutea when the high-dose group was compared to the control group.

There were no deaths in this study. At sacrifice (Day 20) there was a higher incidence of yellow tinged body fat in the treated groups.

The fetal visceral examination revealed hydronephrosis in one mid-dose fetus. Incidental findings included slight dilation of the kidneys and ureters. Skeletal examination of the fetuses revealed anomalies of the ribs and vertebral column in

> one high-dose fetus. Other incidental findings included lagging ossification of the skull, rib cage, vertebral column, pelvic girdle, and extremities and angulated ribs.
>
> Under the conditions of this study, administration of AC 92, 553 to pregnant rats during organogenesis at levels up to and including 500 mg/kg of body weight did not cause abnormalities of fetal development and did not elicit any signs of maternal toxicity.

B. Reviewer's Discussion and Conclusions: An independent review of the individual animal data indicated that the 250 and 500 mg/kg/day dose levels increased the incidence of litters with one or more fetuses having delayed ossification of bones in their extremities (see page 6 above; 4/29, 5/29, 8/28 and 9/30 in the control, low, mid, and high dose groups, respectively). Statistical comparisons of the litter incidences of the two highest dose groups with the control group using Fisher's Exact test provided p values of 0.14 and 0.11 for the 250 and 500 mg/kg/day dose groups, respectively. A statistically significant linear trend was not found when the data were analyzed by the Cochran-Armitige trend test (p=0.099).

A previous review (HED Document No. 008558) indicated that the highest dose tested in this study was adequate. The basis for that conclusion was described as follows:

> The sponsor also indicated that...the oral LD_{50} in Wistar rats was 1250 mg/kg..., in a recent 2-yr chronic/carcinogenicity study (MRID 40174401) in rats, a dose of 5000 ppm (=250 mg/kg/day) produced significant decreases in body weight gain in males and females.

The body weight decrements for female rats in the long-term study were described in HED Document No. 008606 as follows:

> Females in the 5000 ppm group had statistically significant decreases in body weight when compared to controls on Weeks 1 to 100...Mean cumulative body weight gain was decreased in males and females by 10.7 and 25.4 percent, respectively, at 13 weeks when compared to controls.

Although the increased incidences of delayed ossification in the extremities at the two highest doses did not achieve pairwise statistical significance or indicate a clearly dose-related increase, evaluation of a higher dose range would probably be useful in clarifying the apparent association of pendimethalin administration with increased incidences of the delayed ossification noted in the study. In addition, the absence of maternal weight gain decrements after repeated administration of doses as high as 500 mg/kg/day suggests that using the results from the chronic feeding study in rats

to justify the adequacy of dosing in the rat developmental toxicity study is inappropriate.

Comparison of acute toxicity study results may also be misleading since a different strain of rat (Wistar) was used in the acute data referred to in previous reviews, or the vehicle used in the Wistar rat acute study may have been something other than corn oil. Nevertheless, repeated oral doses of 500 mg/kg/day (as compared to an LD_{50} value of 1250 mg/kg) in the developmental study did not cause lethality. Therefore, there is insufficient information available to support characterization of the highest dose tested in the developmental toxicity study as adequate.

The report noted, without including incidence data, that some fetuses exhibited dilated ureters and kidneys. If these observations were made frequently enough to comment on, it is unusual that those results were not included in the report.

DATA EVALUATION REPORT

PENDIMETHALIN

Study Type: SALMONELLA/MAMMALIAN ACTIVATION GENE MUTATION ASSAY

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order 94-15A

Primary Reviewer:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: 4/18/95

Secondary Reviewer:
C. B. Bast, Ph.D., D.A.B.T.

Signature: C B Bast
Date: 4-19-95

Robert H. Ross, M.S., Group Leader

Signature: RHR
Date: 4-19-95

Quality Assurance
S. Chang, M.S.

Signature: S S Chang
Date: 4/19/95

Disclaimer

This DER may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

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[PENDIMETHALIN] Guideline Series 84: MUTAGENICITY

EPA Reviewer: Irving Mauer, Ph.D. Date 05-04-9
Toxicology Branch I (7509C)
EPA Section Head: Marion P. Copley, D.V.M., D.A.B.T. Date 5/9/95
Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay and *Escherichia coli* WP2(uvrA) reverse gene mutation assay(84-2(a))

TOX. CHEM. NO.: 454BB

P.C.CODE: 108501

MRID NUMBER: 431778-01

TEST MATERIAL: Pendimethalin

SYNONYMS: AC92,553; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; Prowl; Herbadox; Stomp

STUDY NUMBER: TD847.501114

SPONSORS: American Cyanamid Company, Agricultural Research Division, P.O. Box 400 Princeton, NJ 08543-0400

TESTING FACILITY: Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD 20850

TITLE OF REPORT: AC 92,553 (Lot No. AC 5213-72A): *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2(uvrA) Reverse Mutation Assay with a Confirmatory Assay

AUTHORS: Richard H.C. San and Valentine O. Wagner, III

REPORT ISSUED: December 3, 1993

EXECUTIVE SUMMARY: In a Salmonella/microsome plate incorporation assay and in an *Escherichia coli* WP2(uvrA) reverse mutation assay (MRID #431778-01), strains TA98, TA100, TA1535, TA1537, TA1538 and WP2(uvrA) were exposed to Pendimethalin at concentrations of 25, 50, 100, 250, 500 and 750 µg/plate, with and without exogenous metabolic activation. Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat livers. The test material was delivered in DMSO.

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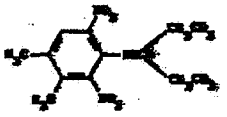
No cytotoxicity was seen at any concentration of Pendimethalin tested. The upper concentration was limited by test material solubility (a precipitate was observed at concentrations of 750 μg/plate and above). Positive and vehicle control values were appropriate. **There was no evidence of an increase number of mutant colonies over solvent control values at any concentration of Pendimethalin tested, either with or without S9 mix.**

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (84-2(a))

A. MATERIALS

1. Test material: Pendimethalin

Description: orange-brown solid
Lot/Batch No.: AC5213-72A
Purity: % a.i. 92.4%
Stability of compound: not given
CAS No.: 40487-42-1 (from The Merck Index, 10th edition)
Structure:



Solvent used: DMSO

2. Control materials

Solvent/final concentration: DMSO/50 μl/plate
Positive:

Non-activation:		
Sodium azide	1.0 μg/plate	TA100, TA1535
2-Nitrofluorene	1.0 μg/plate	TA98, TA1538
9-Aminoacridine	75 μg/plate	TA1537
Methyl Methanesulfonate	1000 μg/plate	WP2(uvrA)
Activation:		
2-Aminoanthracene	1.0 μg/plate	TA98, TA100, TA1535, TA1537, TA1538
	10 μg/plate	WP2(uvrA)

3. Activation

S9 derived from
_X_ Aroclor 1254 _X_ induced _X_ rat _X_ liver
Male Sprague-Dawley rats were used.

S9 mix composition:
10% microsomal enzymes
5 mM glucose-6-phosphate
4 mM β-nicotinamide-adenine dinucleotide phosphate
8 mM $MgCl_2$
33 mM KCl
in a 100 mM phosphate buffer at pH 7.4

4. Test organisms

S. typhimurium strains:
__ TA97 _X_ TA98 _X_ TA100 __ TA102 __ TA104
_X_ TA1535 _X_ TA1537 _X_ TA1538

E. coli strain:
WP2(uvrA)

Properly maintained? YES
Checked for appropriate genetic marker? YES

5. Test compound concentrations used

Non-activated conditions:
Preliminary toxicity test: 100, 250, 750, 1000, 2500, 5000 µg/plate

Mutagenicity test: 25, 50, 100, 250, 500, 750 µg/plate

Activated conditions:
Preliminary toxicity test: 100, 250, 750, 1000, 2500, 5000 µg/plate

Mutagenicity test: 25, 50, 100, 250, 500, 750 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay

X standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification
__ spot test
__ other (describe in a.)

2. Protocol

Plates for the standard plate incorporation assay were prepared as follows, one plate per dose in the preliminary range-finding assay and three per dose in the main mutagenicity assays. In the absence of S9 mix, 100 µl of an overnight culture of tester strain and 50 µl of vehicle, positive control or Pendimethalin were added to 2.5 ml of molten selective top agar (0.8% agar (W/V) and 0.5% NaCl (W/V) supplemented with L-histidine, D-biotin and L-tryptophan to a final concentration of 50 µM each) at 45 ± 2°C. With S9 mix, 500 µl of S9 mix, 100 µl of tester strain and 50 µl of vehicle, positive control or Pendimethalin were added to 2.0 ml of molten selective top agar at 45 ± 2°C. The mixtures were vortexed and overlaid onto the surface of 25 ml of minimal bottom agar (Vogel-Bonner minimal medium E with 1.5% (W/V) agar). When the overlay had solidified, the plates were inverted, incubated at 37 ± 2 °C for 48 hr and then revertant colonies counted. The background lawn of bacteria was also examined to evaluate any cytotoxicity of treatment and any evidence of test material precipitation was noted.

C. REPORTED RESULTS

1. Preliminary cytotoxicity assay

Doses of 100, 250, 750, 1000, 2500 and 5000 µg/plate were use in the range-finding study. No cytotoxicity was seen at these doses; however, a precipitate was seen at doses ≥ 750 µg/plate. Therefore, the upper dose selected for the mutagenicity assays was 750 µg/plate. Results of the preliminary cytotoxicity assay are presented in Appendix Tables 1-6 (MRID No. 431778-01, pp. 17-22) for the six bacterial strains used.

2. Mutagenicity assay

A summary of the results of the mutagenicity assay and the confirmatory assay are given in Appendix Tables 7 and 8 (MRID No. 431778-01, pp. 47-48) respectively. No evidence of a mutagenic response was seen at any dose in any strain with or without S9 mix in either assay. Positive and vehicle control values were appropriate.

D. REVIEWER'S DISCUSSION/CONCLUSIONS

This study conforms to the federal guidelines for conducting a *Salmonella*/microsome reverse mutation assay and a reverse mutation assay at the tryptophan locus in *E. coli* WP2(uvrA). Pendimethalin was tested to a concentration limited by its solubility. The data were consistent within and between experiments and control values were acceptable.

Under the conditions of this study, Pendimethalin did not induce mutations in any of the bacterial strain at any concentration tested, either in the presence or absence of S9 mix.

E. Was test performed under GLPs (is a quality assurance statement present)? YES

F. Appendix attached? NO CBI appendix attached

APPENDIX

TH Review 011796

Page____ is not included in this copy.

Pages 60 through 67 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

✓ FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

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

DATA EVALUATION REPORT

PENDIMETHALIN

Study Type: SALMONELLA/MAMMALIAN ACTIVATION GENE MUTATION ASSAY

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory*
Oak Ridge, TN 37831
Task Order 94-15C

Primary Reviewer:
B. L. Whitfield, Ph.D.

Signature: B L Whitfield
Date: 4/18/95

Secondary Reviewer:
C. B. Bast, Ph.D., D.A.B.T.

Signature: C B Bast
Date: 4-19-95

Robert H. Ross, M.S., Group Leader

Signature: R H R
Date: 4/17/95

Quality Assurance
S. Chang, M.S.

Signature: S S Chang
Date: 4/12/95

Disclaimer

This DER may have been altered by the Health Effects Division subsequent to signing by Oak Ridge National Laboratory personnel.

*Managed by Martin Marietta Energy Systems, Inc., for the U.S. Department of Energy under Contract No. DE-AC05-84OR21400

[PENDIMETHALIN] Guideline Series 84: MUTAGENICITY

EPA Reviewer: Irving Mauer, Ph.D. , Date 05-05-95
Toxicology Branch I (7509C)
EPA Section Head: Marion P. Copley, D.V.M., D.A.B.T. , Date 5/9/95
Toxicology Branch I (7509C)

DATA EVALUATION REPORT

STUDY TYPE: *Salmonella*/mammalian activation gene mutation assay and *Escherichia coli* WP2(uvrA) reverse gene mutation assay(84-2(a))

TOX. CHEM. NO.: 454BB

P.C.CODE: 108501

MRID NUMBER: 431350-06

TEST MATERIAL: Pendimethalin

SYNONYMS: AC92,553; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; Prowl; Herbadox; Stomp

STUDY NUMBER: TC892.501114

SPONSOR: American Cyanamid Company, Agricultural Research Division, P.O. Box 400 Princeton, NJ 08543-0400

TESTING FACILITY: Microbiological Associates, Inc., 9900 Blackwell Road, Rockville, MD 20850

TITLE OF REPORT: AC 92,553: *Salmonella*/Mammalian-Microsome Plate Incorporation Mutagenicity Assay (Ames Test) and *Escherichia coli* WP2(uvrA) Reverse Mutation Assay with a Confirmatory Assay

AUTHORS: Richard H.C. San and Michelle L. Klug

REPORT ISSUED: May 5, 1993

EXECUTIVE SUMMARY: In a Salmonella/microsome plate incorporation assay and in an *Escherichia coli* WP2(uvrA) reverse mutation assay (MRID #431350-06), strains TA98, TA100, TA1535, TA1537, TA1538 and WP2(uvrA) were exposed to Pendimethalin at concentrations of 50, 100, 250, 500 and 750 µg/plate without exogenous metabolic activation and to the same concentrations plus an additional concentration of 25 µg/plate with exogenous metabolic activation. A confirmatory assay tested concentrations of 25, 50, 100,

250, 500 and 750 μg/plate both with and without S9 mix. Preparations for metabolic activation were made from Aroclor 1254 induced male Sprague-Dawley rat livers. The test material was delivered in DMSO.

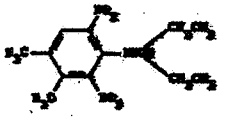
No cytotoxicity was seen at any concentration tested up to 5000 μg/plate. The upper concentration tested was limited by solubility of the test material. Positive and vehicle control values were appropriate. **No evidence of a mutagenic response was seen at any dose in any strain with or without S9 mix in either assay.**

This study is classified as an acceptable study. It satisfies the guideline requirements for a gene mutation study (84-2(a))

A. MATERIALS

1. Test material: Pendimethalin

Description: rust colored powder/ solid
Lot/Batch No.: AC8088-149
Purity: % a.i. 90.7% (provided by sponsor)
Stability of compound: not given
CAS No.: 40487-42-1
Structure:



Solvent used: DMSO

2. Control materials

Solvent/final concentration: DMSO/50 μl/plate
Positive:

Non-activation:		
Sodium azide	1.0 μg/plate	TA100, TA1535
2-Nitrofluorene	1.0 μg/plate	TA98, TA1538
9-Aminoacridine	75 μg/plate	TA1537
Methyl Methanesulfonate	1000 μg/plate	WP2(uvrA)
Activation:		
2-Aminoanthracene	1.0 μg/plate	TA98, TA100, TA1535, TA1537, TA1538
	10 μg/plate	WP2(uvrA)

3. Activation

S9 derived from
_x_ Aroclor 1254 _x_ induced _x_ rat _x_ liver
Male Sprague-Dawley rats were used.

S9 mix composition:
10% microsomal enzymes
5 mM glucose-6-phosphate
4 mM β-nicotinamide-adenine dinucleotide phosphate
8 mM $MgCl_2$
33 mM KCl
in a 100 mM phosphate buffer at pH 7.4

4. Test organisms

S. typhimurium strains:
__ TA97 _x_ TA98 _x_ TA100 __ TA102 __ TA104
_x_ TA1535 _x_ TA1537 _x_ TA1538

E. coli strain:
WP2(uvrA)

Properly maintained? YES
Checked for appropriate genetic marker? YES

5. Test compound concentrations used

Non-activated conditions:
Range-finding test: 100, 250, 500, 750, 1000, 2500, 5000 µg/plate

Mutagenicity test: 50, 100, 250, 500, 750 µg/plate

Activated conditions:

Range-finding test: 100, 250, 500, 750, 1000, 2500, 5000 µg/plate

Mutagenicity test: 25, 50, 100, 250, 500, 750 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay

x standard plate test
__ pre-incubation (__ minutes)
__ "Prival" modification
__ spot test
__ other (describe in a.)

2. Protocol

Plates for the standard plate incorporation assay were prepared as follows, one plate per dose in the preliminary range-finding assay and three per dose in the main mutagenicity assays. In the absence of S9 mix, 100 µl of an overnight culture of tester strain and 50 µl of vehicle, positive control or Pendimethalin were added to 2.5 ml of molten selective top agar (0.8% agar (W/V) and 0.5% NaCl (W/V) supplemented with L-histidine, D-biotin and L-tryptophan to a final concentration of 50 µM each) at 45 ± 2°C. With S9 mix, 500 µl of S9 mix, 100 µl of tester strain and 50 µl of vehicle, positive control or Pendimethalin were added to 2.0 ml of molten selective top agar at 45 ± 2°C. The mixtures were vortexed and overlaid onto the surface of 25 ml of minimal bottom agar (Vogel-Bonner minimal medium E with 1.5% (W/V) agar). When the overlay had solidified, the plates were inverted, incubated at 37 ± 2 °C for 48 hr and then revertant colonies counted. The background lawn of bacteria was also examined to evaluate any cytotoxicity of treatment and any evidence of test material precipitation was noted.

C. REPORTED RESULTS

1. Preliminary cytotoxicity assay

Doses of 100, 250, 500, 750, 1000, 2500 and 5000 µg/plate were use in the range-finding study. No cytotoxicity was seen at these doses; however, a precipitate was seen at doses ≥ 500 µg/plate with TA98, TA100, TA1537 and TA1538 in the presence of S9 mix. A precipitate was seen at doses ≥750 µg/plate with the remaining tester strain/activation combinations. Therefore, the upper dose selected for the mutagenicity assays was either 500 µg/plate or 750 µg/plate as appropriate. Results of the preliminary cytotoxicity assay are presented in Appendix Tables 1 - 6 for the six bacterial strains used.

2. Mutagenicity assay

A summary of the results of the mutagenicity assay and the confirmatory assay are given in Appendix Tables 7 and 8 respectively. In the first mutagenicity assay, the dose range was 50 to 750 µg/plate in the absence of S9 mix and 25 to 750 µg/plate in the presence of S9 mix. In the confirmatory assay the dose range was 25 to 750 µg/plate both with

and without S9 mix. No evidence of a mutagenic response was seen at any dose in any strain with or without S9 mix in either assay. As noted in Appendix Tables 7 and 8, not all strains were tested at 750 μg/plate with S9 mix and, although all strains were tested at 750 μg/plate without S9 mix, none of these plates were counted. Positive and vehicle control values were appropriate.

D. REVIEWER'S DISCUSSION/CONCLUSIONS

This study conforms to the federal guidelines for conducting a *Salmonella*/microsome reverse mutation assay and a reverse mutation assay at the tryptophan locus in *E. coli* WP2(uvrA). Pendimethalin was tested to a concentration limited by its solubility. The data were consistent within and between experiments and control values were acceptable.

As tested in this study, Pendimethalin did not induce reverse mutations at the histidine locus in *S. Typhimurium* tester strains or at the tryptophan locus in *E. coli WP2(uvrA).*

E. Was test performed under GLPs (is a quality assurance statement present)? YES

F. Appendix attached? NO CBI appendix attached

APPENDIX

TRF Review 011796

Page____ is not included in this copy.

Pages 75 through 80 are not included.

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____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

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✓ FIFRA registration data.

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