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

005828

APR - 8 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PROWL (Pendimethalin) - Submission of Data in Response to the Registration Standard (Accession No. 260403)
EPA Registration No. 241-245 (90% Technical)

TOX Chem. 454BB

FROM: William B. Greear, M.P.H.
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Vickie K. Walters/Robert J. Taylor, PM Team 25
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D., Chief
Toxicology Branch
Hazard Evaluation Division (TS-769C)

M. Galley of the American Cyanamid Company, under a cover letter dated October 30, 1985, has submitted several toxicology studies for evaluation as required under the Registration Standard for pendimethalin. The studies are listed below together with their results and "Core" classification.

105 99

Study	Results	Classification
Dermal Sensitization	No sensitization produced	Minimum
Ames Test	Positive	Acceptable
Mammalian Cell Forward Gene Mutation Assay	Negative with S9; Inconclusive without S9	Acceptable with S9; Unacceptable without S9
Chromosomal Aberration	Negative	Acceptable
DNA Repair	Negative	Acceptable

[Although, the Dynamac reviewer recommended that an *in vivo* assay designed to detect gene mutation (e.g., mouse spot test) be conducted, additional mutagenicity studies will not be required at this time because the mutagenicity testing requirements have been satisfied.]

DATA EVALUATION REPORT

005828

1. Subject: Guinea Pig Dermal Sensitization Study

2. Accession No.: 260403

3. Test Material: AC 92,553 (Pendimethalin technical), purity 92.2%
Lot No. AC 3528-129-1, described as a brown-orange crystalline solid

4. Sponsor: American Cyanamid Company
Princeton, NJ 08540

5. EPA Registration No.: 241-245

6. Testing Facility: Biosearch Incorporated
Philadelphia, PA 19134

7. Project No./Date: 85-4639A/August 1, 1985

8. Study Director: B.A. Costello

9. Classification: Minimum

10. Materials and Methods:

Forty male Hartley strain guinea pigs weighing between 408 and 516 g were obtained from Ace Animals, Inc., Boyertown, PA and were allowed to acclimate to laboratory conditions for a period of 14 days. The animals were individually caged in stainless steel cages with elevated wire mesh flooring. The temperature was maintained at 65° to 75 °F and the relative humidity ranged from 30 to 80 percent. A 12-hour light/dark cycle was provided. Wayn· Guinea Pig Diet and tap water were provided ad libitum. An irritation screen was conducted with concentrations of 25%, 50%, and 75% w/v in sterile 0.9% saline and with the test material as supplied. Each concentration was applied to one guinea pig for a 6-hour contact period. No irritation was noted at 24 and 48 hours. Therefore, the main study was conducted with the neat test material. A 4 x 6 cm area was clipped along the back of 36 animals. The backs were clipped weekly through the induction phase and prior to the challenge phase. A 0.43 g portion of the test material was placed on a premoistened (with 0.9% saline) 1-inch square gauze pad which was then applied to an intact skin site on 12 animals. The trunk of each animal was wrapped with plastic wrap which was secured with an elastic bandage. After 6 hours, the wrappings were removed and the site was washed with isopropanol. The site was scored for irritation using the method of Draize at 24 and 48 hours. This sequence was repeated 3 times weekly for 3 weeks for a total of nine applications. After the ninth application, the animals were allowed to rest 2 weeks prior to challenge. The challenge application (0.4 g of the test material) was placed on the right flank for a contact period of 6 hours. The site was scored for irritation at 24 and 48 hours. Twelve animals in the positive control group were treated with a suspension of 0.1% w/v of 1-chloro-2,4-dinitrobenzene in a 50% ethanol:0.9% saline solution. These animals were treated using the same procedure as described for the test animals. An additional 12 animals (naive control group) were maintained in the same manner as the above two groups; however, they remained untreated during the induction phase. These animals were treated with the test material at the same time the test animals were challenged. Observation of the animals for abnormal signs was performed daily for the entire study period. Body weights were determined initially, at weekly intervals, and at termination.

11. Results:

No irritation was observed in guinea pigs treated with the test material during the induction phase or when challenged. No irritation was observed in the naive control

group when challenge was made. Erythema (grade 1) was observed sporadically in guinea pigs in the positive control group at various times during the induction phase. Erythema (up to grade 2) and edema (grade 1) were observed in the majority of the positive control animals when challenged.

12. Conclusion: The test material does not produce sensitization.

13. Classification: Minimum.

14. Justification of Classification:

The study protocol used was a modification of Buehler. Instead of a contact period of 24 hours for the challenge application, a 6-hour period was used. Instead of using 20 test animals, 12 animals were used.

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Pendimethalin (PROWL)

Study / Lab / Date / Study No.	Test Material/ Purity	Genetic End-Point	Accession/ MRID No.	Dose	Conclusions (Evaluation)	Genetic Effects Category	Classification
1. Bacterial/microsome reverse mutation (Ames) test on AC92,553/ American Cyanamid Co./ October 28, 1985/No. 0166.	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Gene mutation in Salmonella and Escherichia	260403	Eight doses ranging from 50 to 5000 µg/plate +/-S9 activation	Positive: a >2-fold dose-related increase in number of revertants, in TA1538 and TA38 +S9. Lowest positive dose was 158 µg/ plate.	1	Acceptable
2. CHO/HGPRT mammalian cell forward gene mutation assay with AC92,553 Lot No. AC 3528-129-1/Pharmakon Research International, Inc./ October 17, 1985/PH314-AC-001-85	AC92,553/92.2%	Gene mutation in Chinese hamster ovary cells	260403	Eight doses ranging from 0.5 to 10 µg/mL +/-S9 activation	Negative +/-S9 activation. Highest assay dose was 10 µg/mL.	1	Acceptable
3. In vitro chromosome aberration analysis in Chinese hamster ovary cells./Pharmakon Research International, Inc./October 17, 1985/PH320-AC-001-85	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Chromosomal aberrations in Chinese hamster ovary cells	260403	Three doses ranging from 5 to 25 µg/mL -S9 and 12 to 100 µg/mL +S9 activation	Negative +/-S9 activation. Highest assay doses were 25 µg/mL with S9 activation and 100 µg/mL nonactivated.	2	Acceptable
4. Rat hepatocyte primary culture/ DNA repair test, AC92,553/ Pharmakon Research, International, Inc./October 25, 1985/PH311-AC-002-85	AC92,553, Lot No. AC 3525-129-1/ 91.2%	Unscheduled DNA synthesis in rat hepatocytes	260403	Seven doses ranging from 30 to 10,000 µg/well; two highest doses 6000 and 10,000 µg/well were cytotoxic	Negative. Highest valid assay doses were 1,500 µg/well and 3,000 µg/ well	3	Acceptable

(Concluded)

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EPA: 68-02-4225
DYNAMAC No. 223-A4
September 15, 1986

DATA EVALUATION RECORD

PENDIMETHALIN (PROWL)

DNA Repair/Rat Hepatocyte

STUDY IDENTIFICATION: Barfknecht, T. R., Naismith, R. W., and Matthews, R. J. Rat hepatocyte primary culture/DNA repair test, AC 92,553. (Unpublished study No. PH 311-AC-002-85 prepared by Pharmakon Research International Inc., Waverly, PA, for American Cyanamid Co., Princeton, NJ; dated October 25, 1985.) Accession No. 260403.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I Cecil Felkner

Date: 9-15-86

005828

1. CHEMICAL: Pendimethalin; AC 92,553; Prowl.

2. TEST MATERIAL: AC 92,553, lot No. AC 3525-129-1, was a brownish orange crystalline solid of 91.2 percent purity and was soluble in DMSO.

3. STUDY/ACTION TYPE: Mutagenicity--Unscheduled DNA synthesis assay in primary rat hepatocytes.

4. STUDY IDENTIFICATION: Barfknecht, T. R., Naismith, R. W., and Matthews, R. J. Rat hepatocyte primary culture/DNA repair test, AC 92,553. (Unpublished study No. PH 311-AC-002-85 prepared by Pharmakon Research International Inc., Waverly, PA, for American Cyanamid Co., Princeton, NJ; dated October 25, 1985.) Accession No. 260403.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 9-15-86

Brenda Worthy, M.T.
Independent Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 9-15-86

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-15-86

William Greear
EPA Reviewer

Signature: William Greear
Date: 9/14/86

Albin Kocialski, Ph.D.
EPA Section Head

Signature: Albin B Kocialski
Date: 3/14/87

7. CONCLUSIONS:

A. Under the conditions of the assay AC 92,553 was negative for unscheduled DNA synthesis (UDS) when tested at levels between 30 and 3000 µg/well. Higher concentrations (6000 and 10,000 µg/well) were cytotoxic. The positive controls 2-acetylaminofluorene (2AAF) and dimethylnitrosamine (DMN) demonstrated the sensitivity of the assay to detect a mutagenic effect.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods:

1. Test Material: The test material was dissolved in dimethylsulfoxide (DMSO), the solvent control, and semilog dilutions were made with the same solvent.

2. Indicator Cells: Primary hepatocytes were obtained from the liver of a 242-g male Fischer 344 rat purchased from Charles River Breeding Laboratories.

3. Cell Preparation: A modification of the basic method of Williams[2] by Kornbrust and Barfknecht[3] was used.

a. Perfusion Technique: The liver was perfused with medium A (Ca^{++}-Mg^{++}-free Hank's balanced salt solution with 0.5 mM EGTA) until blanching began. It was then perfused with Type II collagenase at 100 U/mL in Williams' medium E (WME), pH 7.35, at a rate of 20 mL/minute for 10 minutes. The liver was excised and placed in WME without serum to mechanically release the hepatocytes by combing with a camel hair brush.

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[1] Only items appropriate to this DER have been included.

[2] Williams, G. M. Further improvements in the hepatocyte primary culture DNA repair test for carcinogens. Detection of carcinogenic biphenyl derivatives. Cancer Letters 4(1978): 69-75.

[3] Kornbrust, D. J. and Barfknecht, T. R. Comparison of rat and hamster hepatocyte primary culture/DNA repair assays. Environ. Mutagen. 6(1984): 1-11.

b. Hepatocyte Harvest/Culture Preparation: The detached cells were gently pipetted into centrifuge tubes and allowed to settle for 10 minutes. Viability of the resuspended hepatocytes was determined by trypan blue dye exclusion.

4. Dosage Selection: The test material was screened for toxicity by visual inspection for abnormal cell morphology. It was dissolved in DMSO and 20 semilog dilutions made so that it was tested at levels between 0.006 and 10,000 µg/well; DMSO did not exceed 1 percent in the media. The highest concentration scored for UDS was 3000 µg/well (1500 µg/mL). Six lower concentrations (30, 60, 100, 300, 600, and 1000 µg/well), each in triplicate as well, as vehicle control (DMSO) and positive controls (DMN, 10^{-3} M; 2AAF, 10^{-7} M) were also scored.

5. UDS Assay:

a. Treatment: Viable hepatocytes (10^5) in WME containing 10 percent calf serum were inoculated into 12 well dishes containing plastic coverslips and allowed to attach for 2 hours at 37°C. They were rinsed, and serum-free medium containing nine selected concentrations of test material and 10 µCi/mL [^{3}H]thymidine was added; incubation under CO_2 was continued for 18-20 hours. The cultures were then washed three times with 3 mL volumes of phosphate buffered saline (PBS).

b. UDS Slide Preparation: The attached cells on coverslips were exposed to 1 percent sodium citrate for 10-15 minutes, fixed using three changes of ethanol-acetic acid (3:1) for 30 minutes each, and mounted cell surface up after air drying.

c. Preparation of Autoradiographs/Grain Development: Slides were coated with NBT-2 emulsion in the dark, allowed to dry overnight, and stored at 4°C in desiccated light-proof boxes for 1 week. Slides were then developed in Eastman Kodak D-19, fixed, and stained with Harris alum hematoxylin.

d. Grain Counting: Using coded slides, nuclear grains in 150 cells/dose point were counted. Spontaneous grain counts were quantitated by randomly selecting the highest of three nuclear-sized areas of cytoplasm. "Replicative" DNA synthesis was evidenced by nuclei blackened with grains too numerous to count. Data are presented as mean grains/nucleus from triplicate wells per treatment.

6. Evaluation Criteria: The solvent control should have a net nuclear grain count of 1 or less and should be within the 95 percent confidence limits of historical data (0.18±0.36); the positive control 1 x 10^{-5} M 2AAF should be within one

standard deviation of the mean historical value for this mutagen (28.4±12.4). The test material is considered positive for UDS when a minimum net grain count of five/nucleus is consistently found in triplicate wells and an adequate dose response is observed. It is considered a suspect genotoxic agent if a dose-response is not achieved, and a second experiment may be initiated using more closely bracketed dose levels.

B. Protocol: A detailed protocol was provided (see Appendix A).

12. REPORTED RESULTS:

A. The sponsor analyzed the dosing solutions of the test material and determined that the doses used were from 107.5 to 119.5 percent of the target dose when corrected for a purity of 91.2 percent.

B. The study authors stated that the test material was cytotoxic at 6000 and 10,000 µg/well and that 3000 µg/well was the highest level for which morphological damage could be found; however, the 3000 µg/well dose could be scored for UDS.

C. The test material did not cause an increase in net nuclear grains at 30, 60, 100, 300, 600, 1000, or 3000 µg/well, but was cytotoxic at 6000 and 10,000 µg/well. The positive controls gave the expected response; the mean historical value for 2AAF (1×10^{-5} M) was 28.4±12.4, and in this study 2AAF (1×10^{-7} M) induced 23.9±8.1 net nuclear grains. Table 1 summarizes the results.

D. The viability of the hepatocytes used was 90 percent.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Under the conditions of the assay, the test material AC 92,553 was negative in the rat hepatocyte primary culture/DNA repair test at dose levels of 30, 60, 100, 300, 600, 1000, or 3000 µg/well. This evaluation was based on the inability of the test material to induce a mean nuclear grain count that was five or more than the vehicle control at any level. The two positive controls DMN and 2AAF induced mean net nuclear grains of 22.1 and 23.9, respectively.

B. A quality assurance statement was dated October 25, 1985.

TABLE 1. Autoradiographic Analysis of DNA Repair in the Rat Hepatocyte Primary Culture/DNA Repair Test with AC 92,553

Treatment	Concentrations µg/well	Concentrations µg/mL	Net Nuclear Grains of Triplicate Cultures (X ± S.D.)
Solvent Control			
DMSO (20 µL/well)	---	---	0.1±0.4
Positive Controls			
DMN	1×10^{-3} M+		22.1±7.0*
2AAF	1×10^{-7} M+		23.9±2.1*
Test Material			
AC 92,553	30	15	0.0±0.1
	60	30	0.0±0.2
	100	50	0.1±0.4
	300	150	0.0±0.1
	600	300	0.1±0.4
	1000	500	0.1±0.3
	3000	1500	0.5±1.0
	6000	3000	cytotoxic
	10,000	5000	cytotoxic

*Positive finding. Mean net nuclear grain count of five or more than the vehicle control.

+ = Final concentration in the treatment medium.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the data supported the authors' conclusions and that AC 92,553 did not induce an increase in UDS in rat hepatocytes. The highest dose was adequate, based on the presence of cytotoxicity. Relative survival was not reported for each dose level; however, an adequate number of viable cells was scored at each level.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, pp. 1-10.

APPENDIX A

Protocol

Pendimethalin Tox review 005828

Page_____ is not included in this copy.

Pages 15 through 25 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.

X FIFRA registration data.

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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.

005828

EPA: 68-02-4225
DYNAMAC No. 223-A3
January 9, 1987

DATA EVALUATION RECORD

PENDIMETHALIN (PROWL)

Mutagenicity--Cytogenetics Study in Chinese Hamster Ovary Cells

STUDY IDENTIFICATION: San Sebastiam, J. R., Naismith, R. W., and Matthews, R. J. In vitro chromosome aberration analysis in Chinese hamster ovary (CHO) cells. (Unpublished study No. PH 320-AC-001-85 prepared by Pharmakon Research International, Inc., Waverly, PA, for American Cyanamid Company, Princeton, NJ; dated October 17, 1985.) Accession No. 260403.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-9-87

1. CHEMICAL: Pendimethalin; AC 92,553; Prowl.

2. TEST MATERIAL: AC 92,553, lot No. AC 3528-129-1, was described as a brownish orange solid with a purity of 92.9%.

3. STUDY/ACTION TYPE: Mutagenicity--Cytogenetics study in Chinese hamster ovary cells.

4. STUDY IDENTIFICATION: San Sebastian, J. R., Naismith, R. W., and Matthews, R. J. In vitro chromosome aberration analysis in Chinese hamster ovary (CHO) cells. (Unpublished study No. PH 320-AC-001-85 prepared by Pharmakon Research International, Inc., Waverly, PA, for American Cyanamid Company, Princeton, NJ; dated October 17, 1985.) Accession No. 260403.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 1-9-87

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-9-87

6. APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: 1-9-87

William Greear, Ph.D.
EPA Reviewer

Signature: William B Greear
Date: January 3 '87

Albin Kocialski, Ph.D.
EPA Section Head

Signature: Albin B Kocialski
Date: 3/14/87

7. **CONCLUSIONS:**

A. Under the conditions of the assay, three doses of AC 92,553, ranging from 5 to 25 µg/plate, -S9, and 12.5 to 100 µg/mL, +S9, did not induce an increase in chromosomal aberrations in Chinese hamster ovary (CHO) cells. The positive controls N-methyl-N-nitro-N-nitrosoguanidine (MNNG) at 2 µg/mL and N-nitrosodimethylamine (DMN) at 1000 µg/mL demonstrated the sensitivity of the assay to detect a clastogenic effect.

B. The study is acceptable.

Items 8 through 10—see footnote 1.

11. **MATERIALS AND METHODS (PROTOCOLS):**

A. Materials and Methods:

1. Test Material: AC 92,553, lot No. AC 3528-129-1, was described as a brownish-orange solid with a purity of 92.2%. The test material was dissolved in dimethylsulfoxide (DMSO), the solvent control.

2. Cell Line: CHO cells, strain K1, subclone K1-BH4, were obtained from Dr. Abraham W. Hsie, Oak Ridge, TN. Stock cultures were maintained frozen at -80°C. Both stock and working cultures were routinely checked for mycoplasma contamination, stable karyotype, and polyploid index. Cells used in this assay were harvested from logarithmic-growing cultures, trypsinized, resuspended in Ham/F12 +5% fetal calf serum (F12FCM), dispensed at a density of 8×10^5 cells/75 cm^2 tissue culture flasks, and incubated for 16-24 hours at 37°C.

3. S9 Fraction: The S9 fraction was prepared from the liver of a rat (strain not specified) induced with Aroclor 1254. The S9 mix contained the appropriate cofactor and 10% S9 fraction.

4. Positive Controls: MNNG at 2 µg/mL and DMN at 1000 µg/mL were used as the nonactivated and S9-activated positive controls, respectively.

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[1] Only items appropriate to this DER have been included.

5. Preliminary Cytotoxicity Test: Logarithmic-growing cell cultures were untreated or exposed to 10 doses of the test material or to the solvent controls for 5 hours with S9 activation and for 18 hours without S9 activation. After treatment, cultures were washed, resuspended in fresh F12FCM, and reincubated for 16.5 hours with S9 and for 2.5 hours without S9 at 37°C in 5% CO_2 at ≥90% humidity. During the last 2 hours of incubation, colcemid was added to arrest cells in metaphase. After incubation, cells were processed, and slides were prepared and analyzed for mitotic index (MI); a total of 500 metaphases per treatment was examined. The dose that produced a 50% mitotic depression was selected as the highest dose for the CHO cytogenetic assay.

6. Cytogenetics Assay:

   a. Treatment: Duplicate cell cultures were untreated or exposed to three doses of the test material or solvent or positive controls for 5 hours with S9 activation and for 8 or 19 hours without S9 activation. To terminate exposure, cells were washed with Saline G and refed with F12FCM and reincubated. Colcemid was added to each culture 2-3 hours prior to cell harvest.

   b. Harvest and Slide Preparation: Cells were harvested at 11 hours posttreatment with or without S9 activation and at 21.5 hours posttreatment with or without S9 activation using mitotic shake-off. Cells were washed in hypotonic KCl and fixed with methanol:glacial acetic acid (3:1). Fixed cells were dropped onto slides, air dried, stained in 3% Giemsa, and mounted. All slides were randomly coded prior to analysis.

   c. Metaphase Analysis: One hundred metaphase cells (50/flask) per treatment group were scored for chromosomal aberrations, i.e., chromatid breaks, interchanges, rings, complex rearrangements, or for cells with >10 aberrations. Gaps were counted, but were not included in the final analysis.

7. Evaluation Criteria: A test material was considered positive if it caused a statistically significant increase in aberrations/cell in at least one dose level and in the number of aberrant cells when compared to the solvent control and/or a dose-related increase was demonstrated.

8. Statistical Analysis: Data were evaluated for significance using Chi-square and a one-tailed t-test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Test: AC 92,553 was assayed at doses of 2, 20, 40, 60, 80, 100, 125, 150, 175, and 200 µg/mL in the presence or absence of S9 activation. At dose levels from 20 to 200 µg/mL, a discoloration (orange color) of the medium was observed. Test material precipitation was also noted at doses ≥125 µg/mL after adding the test material to the medium. Cells did not survive at doses ≥150 µg/mL with or without S9 activation. Cell morphology was abnormal at doses of 100 and 125 µg/mL, +S9, and at doses from 40 to 125 µg/mL, -S9.

A dose-related depression was observed in the MI in the nonactivated assay, 15% at 2 µg/mL to 80% at 125 µg/mL with a 60% decrease at the 100-µg/mL dose. In the presence of S9 activation, no appreciable MI depression was noted.

The dose levels selected for cytogenetic analysis were 10, 35, and 100 µg/mL, -S9, and 12.5, 45, and 125 µg/mL, +S9.

B. Cytogenetic Assay: In the initial assay without S9 activation at the 35- and 100-µg/mL doses, the cytotoxic effect resulted in too few metaphases for analysis at either one or both harvest intervals. In the S9-activated assay, the highest dose (125 µg/mL) was cytotoxic at the late harvest interval. Therefore, the assay was repeated.

The doses selected for the repeat assay were 4, 20, and 40 µg/mL, -S9, and 12.5, 45, and 100 µg/mL, +S9.

In the absence of S9 activation, the test material was cytotoxic, resulting in too few metaphases for analysis in both harvest intervals at the 40 µg/mL dose and in the early harvest interval at the 20 µg/mL dose. Doses selected for the S9 activated assay were all scorable.

A second repeat assay was performed at nonactivated doses of 1, 5, 10, 15, 20, and 25 µg/mL. There was an adequate number of scorable metaphases observed at all dose levels.

The dose levels scored for chromosomal aberrations were 5, 15, and 25 µg/mL without S9 activation and 12.5, 45, and 100 µg/mL with S9 activation. Compared to the solvent control, no significant increases were observed in the frequency of chromosomal aberrations. The positive controls, MNNG and DMN, induced significant increases in aberrations/cell and in the number of aberrant metaphases, demonstrating the sensitivity of the assay.

Representative results are presented in Table 1 and 2.

TABLE 1. Representative Results from the In Vitro CHO Cytogenetic Assay with AC 92,553

Substance		S9 Activation	Harvest Interval (hr)	No. of Metaphases Examined	No. of Aberrant Cells	Total Aberrants per Cell ±SD
Solvent Control						
Dimethylsulfoxide		-	11	100	4	0.04±0.20
		-	21.5	100	1	0.01±0.10
		+	11	100	2	0.02±0.14
		+	21.5	100	0	0.00±0.00
Positive Controls[a]						
MNNG	2 µg/mL	-	21.5	100	84*	2.66±2.51*
DMN	1000 µg/mL	+	21.5	100	55*	0.84±0.98*
Test Material						
AC 95,553	25 µg/mL[b]	-	11	100	5	0.06±0.28
		-	21.5	100	0	0.00±0.00
	100 µg/mL[c]	+	11	100	5	0.05±0.219
		+	21.5	100	5	0.05±0.219

[a]MNNG = N-methyl-N-nitro-N-nitrosoguanidine.

DMN = N-nitrosodimethylamine.

*Statistically significant from control value ($p \leq 0.05$).

[b]Highest dose tested; with lower doses (5 and 15 µg/mL) the results were comparable to solvent control.

[c]Highest dose tested; with lower doses (12.5 and 45 µg/mL) the results were comparable to solvent control.

TABLE 2. Representative Types of Aberrations[a] Observed in the In Vitro CHO Cytogenetic Assay with AC 92,553

Substance	S9 Activation	Harvest (hr)	Chromatid Aberrations Exchanges					Chromosome Aberrations					Total Aberrations
			Gaps	Del	Interch	Intrach	Trir	Rb	Del	Ring	Dic	Misc	
Solvent Control													
Dimethylsulfoxide	-	11	3	1	0	0	0	0	3	0	0	0	4
	-	21.5	1	0	0	0	0	1	0	0	0	0	1
	+	11	2	2	0	0	0	0	0	0	0	0	2
	+	21.5	1	0	0	0	0	0	0	0	0	0	0
Positive Controls[b]													
MNNG 2 µg/mL	-	21.5	15	63	78	34	33	6	6	1	5	4	266*
DMN 1000 µg/mL	+	21.5	9	27	23	12	11	3	8	0	0	0	84*
Test Material													
AC95,553 25 µg/mL[c]	-	11	8	2	0	0	0	0	2	0	2	0	6
	-	21.5	2	0	0	0	0	0	0	0	0	0	0
100 µg/mL[d]	+	11	24	4	0	0	0	0	0	0	1	0	5
		21.5	6	4	0	0	0	0	1	0	0	0	5

*Statistically significant from control value ($p \leq 0.05$).

[a]Aberration: Del = Deletions; Interch = Interchange; Intrach = Intrachange; Trir = Triradical; Rb = Robertsonian; Dic = Dicentric; Misc = Miscellaneous (inversions, translocations, shattered chromosomes or multiple aberrations).

[b]MNNG = N-methyl-N-Nitro-N-nitrosoguanidine
DMN = N-nitrosodimethylamin

[c]Highest dose tested; the results of lower doses (5 and 15 µg/mL) were comparable to the solvent control.

[d]Highest dose tested; the results of lower doses (12 and 45 µg/mL) were comparable to the solvent control.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "Based on the findings of this assay, AC 92,553, Lot #AC 3528-129-1, was judged negative in the In Vitro Chromosome Aberration Assay in Chinese Hamster Ovary (CHO) cells at any dose level tested both with and without metabolic activation under the experimental conditions of this laboratory."

B. A quality assurance statement was signed and dated October 1, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study authors interpreted the data correctly and that AC 92,553, lot No. AC 3528-129-1, did not cause an increase in chromosomal aberration frequency in CHO cells. The positive controls, DMN and MNNG, demonstrated the sensitivity of the assay to detect a clastogenic response with and without metabolic activation.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-8; Appendix B, Protocol, CBI (Appendix) pp. 1-8.

APPENDIX A

Materials and Methods

Pendimethalin Tox review 005828

Page_____ is not included in this copy.

Pages 35 through 49 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.

X FIFRA registration data.

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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.

005828

EPA: 68-02-4225
DYNAMAC No. 223A-2
October 7, 1986

DATA EVALUATION RECORD

PENDIMETHALIN (PROWL)

Mutagenicity--CHO/HGPRT Point Mutation Assay

STUDY IDENTIFICATION: Stankowski, L. F., Naismith, R. W., and Matthews, R. J. CHO/HGPRT mammalian cell forward gene mutation assay with AC 92,553 Lot #AC3528-129-1. (Unpublished study No. PH314-AC-0C1-85 prepared by Pharmakon Research International, Inc., Waverly, PA, for American Cyanamid Company, Princeton, NJ; dated October 17, 1985.) Accession No. 260403.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 11-7-86

50

1. CHEMICAL: Pendimethalin; AC, 92,533; Prowl.

2. TEST MATERIAL: AC 92,553, lot No. AC 3528-129-1, was described as a brownish-orange crystalline solid with a purity of 92.2%.

3. STUDY/ACTION TYPE: Mutagenicity--CHO/HGPRT point mutation assay.

4. STUDY IDENTIFICATION: Stankowski, L. F., Naismith, R. W., and Matthews, R. J. CHO/HGPRT mammalian cell forward gene mutation assay with AC 92,553 Lot #AC3528-129-1. (Unpublished study No. PH314-AC-001-85 prepared by Pharmakon Research International, Inc., Waverly, PA, for American Cyanamid Company, Princeton, NJ; dated October 17, 1985.) Accession No. 260403.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: October 7, 1986

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 10-7-86

6. APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: Oct. 7, 1986

William Greear, M.S.
EPA Reviewer

Signature: William B. Greear
Date: 10/8/86

Albin Kocialski, Ph.D.
EPA Section Head

Signature: William B Kocialski
Date: 3/11/87

7. CONCLUSIONS:

A. Under the conditions of the assay, eight S9-activated doses of AC 92,553 ranging from 10 to 80 µg/mL, did not induce an increase in mutant frequency in CHO/HGPRT cells. The positive controls, ethylmethanesulfonate and dimethylnitrosamine, demonstrated the sensitivity of the assay to detect a mutagenic response. However, the highest dose (10 µg/mL) tested without S9 activation did not elicit an appropriate cytotoxic response, therefore, the dose range selected to determine the mutagenic potential of AC 92,553 -S9 activation was inadequate.

B. The study is acceptable with S9 activation; however, unacceptable in the absence of S9 activation.

8. RECOMMENDATION:

It is recommended that the nonactivated mutation assay be repeated with a dose range that demonstrates the test material's limit of cytotoxicity.

Items 9 and 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for complete details.)

1. Test Materials: AC 92,553, lot No. AC 3528-129-1, was described as a brownish-orange crystalline solid with a purity of 92.2%. The test material was dissolved in dimethylsulfoxide (DMSO), the solvent control.

2. Cell Line: The Chinese hamster ovary (CHO) cells used in the assay were of a BH_4 subclone from the CHO-K_1 cell line developed by Dr. Abraham Hsie. Stock cultures were maintained frozen at -60°C. Both frozen and working cultures were periodically analyzed for mycoplasma contamination and karyotype stability. Sixteen to 24 hours prior to initiation of the assay, cells were grown in Ham's F12 nutrient medium supplemented with 5% dialyzed and heat-inactivated fetal bovine serum (F12FCM5).

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[1]Only items appropriate to this DER have been included.

3. S9 Fraction: The S9 fraction was prepared from the liver of a male Sprague-Dawley rat induced with Aroclor 1254; the S9 mix contained 10% S9 fraction and the appropriate cofactors.

4. Positive Controls: The positive controls used were ethylmethanesulfonate (200 µg/mL) and dimethylnitrosamine (100 µg/mL).

5. Preliminary Cytotoxicity Assay: Cells in exponential growth, seeded at 0.8 to 1.2x10^6 cells/25 cm^2 flask, were washed twice and refed with serum-free Ham's F12 prior to treatment. Duplicate cultures were exposed for 5 hours to 10 doses of the test material ranging from 0.0833 to 2500 µg/mL with or without S9 activation; incubation was at 37°C in 5% CO_2/air with >90% humidity. After the 5-hour incubation, cultures were washed and refed with F12FCM5. Cultures were reincubated for 19 hours under standard conditions.

   Following the 19-hour incubation period, cultures were washed and harvested. The cell number was determined for each culture using an automatic counter. An aliquot of each cell culture was diluted and plated in triplicate at 200 cells/plate. Plates were incubated for 7 days, and colonies were fixed, stained, and counted. Survival frequencies were calculated, and doses were selected for the mutation assay. Test material doses were expected to yield 10-100% survival.

6. CHO Mutation Assay:

   a. Treatment: Triplicate cell cultures were prepared and treated with appropriate levels of test material and solvent or positive controls with or without S9 activation. After a 5-hour incubation cells were washed, refed, and incubated. Following 19 hours of incubation, cells were plated at 200 cells/plate for cytotoxicity assessment; the remaining cell cultures, seeded at 1 x 10^6 cells/plate, were subcultured for the mutation expression period.

   b. Mutation Expression Period: Cells were subcultured on days 3 and 6 of the 8-day expression period. Cells were harvested and the cell density was determined. An aliquot of 1 x 10^6 cells were then placed on 100-mm plates.

   c. Mutation Selection: Selection of 6-thioguanine-resistant mutants (TG^r) was accomplished by plating 2 x 10^5 cells (five replicates) from each treatment group onto hypoxanthine-free F12FCM5 containing 12.5 µM TG. Cloning efficiency (at selection) was assessed by plating 200 cells/plate (3 replicates) in medium free of TG. All plates were incubated for 7-10 days, and colonies were fixed, stained, and counted. The mutant frequency (MF) was calculated and expressed as the number of TG^r mutants/10^6 clonable cells.

7. Evaluation Criteria: A test material was considered positive if the average MF at a given dose was significant ($p < 0.5$) by the Student t-test or if it induced a significant ($p = < 0.5$) dose-related response using the z-test.

B. Protocol: See Appendix B.

12. REPORTED RESULTS:

A. Preliminary Cytotoxicity Assay: AC 92,553 was assayed at 0.0833, 0.25, 0.833, 2.5, 8.33, 25, 83.3, 250, 833, and 2500 µg/mL. Initial cell survival was 88.9% at 25 µg/mL, +S9, and 56% at 8.33 µg/mL, -S9. Due to the cytotoxic effect a repeat assay was performed.

The test material was assayed at 0.5, 1.0, 2.5, 5.0, 7.5, 10, 12.5, 15, 17.5, 20, 22.5, and 25 µg/mL without S9 and at 12.5, 25, 37.5, 50, 62.5, 75, 87.5, and 100 µg/mL with S9 activation. Cell survival was 31.8% at 10 µg/mL without S9 activation; doses $\geq$12.5 µg/mL were discarded due to extreme cytotoxicity. In the presence of S9 activation, cell survival was 47.1% at 62.5 µg/mL; doses $\geq$75 µg/mL were discarded due to extreme cytotoxicity. Based on these results the dose levels selected for the mutation assay were 0.5, 1.0, 2.5, 5.0, 6.25, 7.5, 8.75, and 10 µg/mL, -S9, and 10, 20, 30, 40, 50, 60, 70, and 80 µg/mL, +S9.

B. Mutation Assay: Nonactivated test material at 10 µg/mL resulted in relative cell survival of 92% and an average cloning efficiency of 67.6%. Test material at 80 µg/mL activated with S9 gave a relative survival of 40.4% and a cloning efficiency of 62.8%.

The average MF of the solvent control with and without S9 activation was $10/10^6$ and $7.4/10^6$ clonable cells, respectively. Without S9 activation, the MF of the test material ranged from 24.2 to $22.9/10^6$ clonable cells at doses from 0.5 to 10 µg/mL. With S9 activation, the MF of the test material ranged from 10.2 to $7.9/10^6$ clonable cells at doses from 10 to 80 µg/mL.

No statistically significant or dose-related increases were observed in the MF

Representative results are presented in Table 1.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The authors concluded that "These results indicate test article AC 92,553 was negative in the CHO/HGPRT Mammalian Cell Forward Gene Mutation Assay under the conditions, and according to the criteria, of the test protocol."

TABLE 1. Representative Results from the CHO/HGPRT Point Mutation Assay with AC 92,553

Substance	S9 Activation	Initial[a] Relative Survival (%)	Cloning[a] Efficiency (%)	Mutant Frequency[a] Mutants/10^6 Clonable Cells
Solvent Control				
Dimethylsulfoxide	-	102.9	66.9	10.9
	+	96.7	67.5	7.4
Positive Controls				
Ethylmethanesulfonate 200 µg/mL	-	79.4	61.2	263.9*
Dimethylnitrosamine 100 µg/mL	+	27.0	46.8	197.6*
Test Material				
AC 92,553				
10 µg/mL[b]	-	92.4	67.6	22.9
80 µg/mL[c]	+	40.4	62.8	7.9

[a]Average of triplicate plates.

[b]Highest dose tested; with lower doses (0.5, 1, 2.5, 5, 6.25, 7.5, and 8.75 µg/mL) the results were comparable to solvent control.

[c]Highest dose tested; with lower doses (10, 20, 30, 40, 50, 60, and 70 µg/mL) the results were comparable to solvent control.

*Significant increase over the solvent control value ($p < 0.05$).

B. A quality assurance statement was signed and dated October 9, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that AC 92,553 assayed at eight doses ranging from 10 to 80 µg/mL with S9 activation did not induce gene mutation in CHO/HGPRT cells. The positive controls ethylmethanesulfonate at 200 µg/mL and dimethylnitrosamine at 100 µg/mL adequately demonstrated the sensitivity of the assay to detect a mutagenic response.

However, because the test material at the highest dose (10 µg/mL) tested without S9 activation failed to elicit an appropriate cytotoxic response, we assess that the dose range selected to determine the mutagenic potential of AC 92,553 was inadequate.

The authors stated that based on the cytotoxicity assays, the doses chosen for the mutation assay were expected to yield 10-100% survival and as stipulated under the authors' criteria for an acceptable assay that at least one concentration of the test material should produce cytotoxicity of >50%, i.e., <50% relative survival (%RS).

In the preliminary cytotoxicity assay, the test material at a dose of 8.33 µg/mL resulted in 56% RS and in the repeat cytotoxicity assay at 10 µg/mL the %RS was 31.8%. However, in the mutation assay the test material at the highest dose (10 µg/mL) tested resulted in 92.4% RS or only 7.6% cytotoxicity. Perhaps the dissimilarities between the two cytotoxicity assays and the mutation assay were due to dilution errors. Therefore, based on the reported data, the mutation assay with S9 activation should be repeated.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 2-6; Appendix B, Protocol, CBI (Appendix) pp. 1-9.

APPENDIX A

Materials and Methods

Pendimethalin Tox review 005828

Page_____ is not included in this copy.

Pages 58 through 73 are not included.

The material not included contains the following type of information:

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X FIFRA registration data.

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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.

005828

EPA: 68-02-4225
DYNAMAC No. 223-A5
January 22, 1987

OVERVIEW

PENDIMETHALIN (PROWL)

Mutagenicity

STUDY IDENTIFICATION: Overview on the Mutagenicity of the Pesticide Pendimethalin (Prowl).

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: [signature]

Date: 1-22-87

005828

EPA: 68-02-4225
Dynamac No. 223-A5
January 22, 1987

OVERVIEW

PENDIMETHALIN (PROWL)

Mutagenicity

STUDY IDENTIFICATION: Overview on the Mutagenicity of the Pesticide Pendimethalin (Prowl).

REVIEWED BY:

I. Cecil Felkner, Ph.D.
Principal Author
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-22-87

APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: 1-22-87

William Greear, M.P.H.
EPA Reviewer

Signature: William B Greear
Date: 1/29/87

Albin Kocialski, Ph.D.
EPA Section Head

Signature: Albin B. Kocialski
Date: 3/14/87

TEST CHEMICALS: AC 92,553, lot No. AC 352-129-1 with a purity of 92.2%.

STUDY/ACTION TYPE: Overview--Registration Action.

MUTAGENICITY OVERVIEW ON THE PESTICIDE PENDIMETHALIN (PROWL):

Introduction: Under FIFRA Guideline Subdivision F: Pesticide Assessment Guidelines: Hazard Evaluation--Human and Domestic Animals, dated 11-30-82, an overview (Section 80-1) is required for the various subdivisions of toxicology. "This subdivision details the toxicity data recommended to support the registration of pesticide products" and should meet the requirements of good laboratory practice (40 CFR Part 160), if applicable.

For each test substance, bioassays must be performed to assess its "potential to affect the qualitative or quantitative integrity of human genetic material." A battery of tests to assess mutagenicity is therefore required with the objectives of:

1. Detecting, with great sensitivity, the capacity of a test material to alter cellular genetic material.

2. Determining the relevance of genetic alterations to mammals.

3. Incorporating positive genetic findings into the risk assessments for heritable effects, carcinogenicity, and possibly other health end-points.

There are three categories of genetic effects that must be addressed by the test battery. These categories are:

1. Gene mutations.

2. Structural chromosomal aberrations.

3. Other mutagenic mechanisms (e.g., direct DNA damage, microtubule/spindle fiber inhibition) as deemed appropriate for the test material.

Mutagenicity data as required by 40 CFR Section 158.135 are to be submitted to support the registration of each manufacturing-use product and of certain end-use products. The assays are to be performed with the technical grade of each active ingredient in the product. The product should be tested in nonactivated and metabolically activated *in vitro* assays and should also be assayed using *in vivo* mammalian systems with all appropriate positive and negative controls; Subpart F of the Toxic Substance Control Act (Fed. Register Vol. 50, No. 188, 9-27-85) provides the rules and regulations for conducting the various assays to determine genetic toxicity.

SUMMARY OF STUDY EVALUATIONS:

Category 1: Gene Mutation. Two studies in this category, one using the bacterial/microsome reverse mutation assay in S. typhimurium (study No. 1, Table 1) and one using the CHO/HGPRT mammalian cell forward gene mutation assay (study No. 2, Table 1) were conducted with AC 92,553 of technical grade. Both were classified as acceptable; however, the test material was mutagenic with S9 activation at doses from 158 through 5,000 µg/plate in the bacterial assay with strains TA98 and TA1538 (Ames test) whereas it was nonmutagenic at all eight doses ranging from 0.5 to 10 µg/mL in the CHO/HGPRT assay. These studies fulfill the category 1 in vitro testing requirements.

Category 2: Structural Chromosomal Aberrations. One in vitro study was conducted using Chinese hamster ovary cells (study No. 3, Table 1). The test material, technical AC 92,553 was non-clastogenic at nonactivated doses of 5 to 25 µg/mL and at S9 activated doses of 12 to 100 µg/mL, respectively. The study was classified as acceptable and fulfills the category 2 in vitro testing requirements.

Category 3: Other Mutagenic Mechanisms. One study was conducted in vitro using rat primary hepatocytes (study No. 4, Table 1). The test material, technical AC 92,553, did not cause unscheduled DNA synthesis (DNA repair) at doses ranging from 30 to 10,000 µg/well or 15 to 5,000 µg/mL. However, cytotoxicity was excessive at the two highest dose levels; therefore, the highest negative responses were considered to be 1,500 µg/mL and 3,000 µg/well, respectively.

CONCLUSIONS:

All of the in vitro studies conducted on technical AC 92,553 (Pendimethalin) were acceptable; therefore all in vitro testing requirements have been met by the studies. The test material did not cause chromosomal aberrations or unscheduled DNA synthesis under the conditions of the studies, nor did it cause gene mutation in the CHO/HGPRT mammalian assay. However, it induced a dose-related increase in two Salmonella strains (TA1538 and TA98); hence, it can be classified as a frameshift mutagen. There were no in vivo assays conducted; therefore, a data gap in mutagenicity exists. Furthermore, the positive response in Salmonella and negative response in CHO/HGPRT cells are in contrast; therefore, the gene mutation response must be further tested.

RECOMMENDATIONS:

It is recommended that in vivo assays designed to detect gene mutations (e.g., morse spot test) should be conducted. Additional in vitro mammalian cell culture assays (e.g., mouse lymphoma assay) should be conducted using a different system so that the question of gene mutation potential in mammalian cells can be resolved.

TABLE 1. One-Liner Summary Table of Mutagenicity Studies with Pendimethalin (PROWL)

Study / Lab / Date / Study No.	Test Material/ Purity	Genetic End-Point	Accession/ MRID No.	Dose	Conclusions (Evaluation)	Genetic Effects Category	Classification
1. Bacterial/microsome reverse mutation (Ames) test on AC92,553/ American Cyanamid Co./ October 28, 1985/No. 0166.	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Gene mutation in Salmonella and Escherichia	260403	Eight doses ranging from 50 to 5000 µg/plate +/-S9 activation	Positive: a >2-fold dose-related increase in number of revertants, in TA1538 and TA38 +S9. Lowest positive dose was 158 µg/ plate.	1	Acceptable
2. CHO/HGPRT mammalian cell forward gene mutation assay with AC92,553 Lot No. AC 3528-129-1/Pharmakon Research International, Inc./ October 17, 1985/PH314-AC-001-85	AC92,553/92.2%	Gene mutation in Chinese hamster ovary cells	260403	Eight doses ranging from 0.5 to 10 µg/mL +/-S9 activation	Negative +/-S9 activation. Highest assay dose was 10 µg/mL.	1	Acceptable
3. In vitro chromosome aberration analysis in Chinese hamster ovary cells./Pharmakon Research International, Inc./October 17, 1985/PH320-AC-001-85	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Chromosomal aberrations in Chinese hamster ovary cells	260403	Three doses ranging from 5 to 25 µg/mL -S9 and 12 to 100 µg/mL +S9 activation	Negative +/-S9 activation. Highest assay doses were 25 µg/mL with S9 activation and 100 µg/mL nonactivated.	2	Acceptable
4. Rat hepatocyte primary culture/ DNA repair test, AC92,553/ Pharmakon Research, International, Inc./October 25, 1985/PH311-AC 002 85	AC92,553, Lot No. AC 3525 129-1/ 91.2%	Unscheduled DNA synthesis in rat hepatocytes	260403	Seven doses ranging from 30 to 10,000 µg/well; two highest doses 6000 and 10,000 µg/well were cytotoxic	Negative. Highest valid assay doses were 1,500 µg/mL/well and 3,000 µg/ well	3	Acceptable

(Concluded)

005828

TABLE 1. One Liner Summary Table of Mutagenicity Studies with Pendimethalin (PROWL)

Study / Lab / Date / Study No.	Test Material/ Purity	Genetic End-Point	Accession/ MRID No.	Dose	Conclusions (Evaluation)	Genetic Effects Category	Classification
1. Bacterial/microsome reverse mutation (Ames) test on AC92,553/ American Cyanamid Co./ October 28, 1985/No. 0166.	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Gene mutation in Salmonella and Escherichia	260403	Eight doses ranging from 50 to 5000 µg/plate +/-S9 activation	Positive: a >2-fold dose-related increase in number of revertants, in TA1538 and TA38 +S9. Lowest positive dose was 158 µg/ plate.	1	Acceptable
2. CHO/HGPRT mammalian cell forward gene mutation assay with AC92,553 Lot No. AC 3528-129-1/Pharmakon Research International, Inc./ October 17, 1985/PH314-AC-001-85	AC92,553/92.2%	Gene mutation in Chinese hamster ovary cells	260403	Eight doses ranging from 0.5 to 10 µg/mL +/-S9 activation	Negative +/-S9 activation. Highest assay dose was 10 µg/mL.	1	Acceptable
3. In vitro chromosome aberration analysis in Chinese hamster ovary cells./Pharmakon Research International, Inc./October 17, 1985/PH320-AC-001-85	AC92,553, Lot No. AC 3528-129-1/ 92.2%	Chromosomal aberrations in Chinese hamster ovary cells	260403	Three doses ranging from 5 to 25 µg/mL -S9 and 12 to 100 µg/mL +S9 activation	Negative +/-S9 activation. Highest assay doses were 25 µg/mL with S9 activation and 100 µg/mL nonactivated.	2	Acceptable
4. Rat hepatocyte primary culture/ DNA repair test, AC92,553/ Pharmakon Research, International, Inc./October 25, 1985/PH311-AC-002-85	AC92,553, Lot No. AC 3525-129-1/ 91.2%	Unscheduled DNA synthesis in rat hepatocytes	260403	Seven doses ranging from 30 to 10,000 µg/well; two highest doses 6000 and 10,000 µg/well were cytotoxic	Negative. Highest valid assay doses were 1,500 µg/well and 3,000 µg/ well	3	Acceptable

(Concluded)

005828

EPA: 68-02-4225
DYNAMAC No. 223-A1
September 12, 1986

DATA EVALUATION RECORD

PENDIMETHALIN (PROWL)

Mutagenicity--Reverse Mutation Assay with Salmonella and Escherichia

STUDY IDENTIFICATION: Allen, J. S., Fine, B. C., Johnson, E., Panfili, J., and Caterson, C. R. Bacterial/microsome reverse mutation (Ames) test on AC 92,553 (Lot AC 3528-129-1). (Unpublished study No. 0166 prepared and submitted by American Cyanamid Co., Agricultural Research Division, Princeton, NJ; dated October 28, 1985.) Accession No. 260403.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 9-12-86

1. CHEMICAL: Pendimethalin; AC 92,553; Prowl; N-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitro-benzenamine.

2. TEST MATERIAL: AC 92,553, lot No. AC 352-129-1, CAS No. 40487-42-1, had a purity of 92.2%; no physical description was reported.

3. STUDY/ACTION TYPE: Mutagenicity--Reverse mutation assay with Salmonella and Escherichia.

4. STUDY IDENTIFICATION: Allen, J. S., Fine, B. C., Johnson, E., Panfili, J., and Caterson, C. R. Bacterial/microsome reverse mutation (Ames) test on AC 92,553 (Lot AC 3528-129-1). (Unpublished study No. 0166 prepared and submitted by American Cyanamid Co., Agricultural Research Division, Princeton, NJ; dated October 28, 1985.) Accession No. 260403.

5. REVIEWED BY:

Brenda Worthy, M.T.
Principal Reviewer
Dynamac Corporation

Signature: Brenda Worthy
Date: 9-12-86

I. Cecil Felkner, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 9-12-86

6. APPROVED BY:

William L. McLellan, Ph.D.
Genetic Toxicology
Technical Quality Control
Dynamac Corporation

Signature: William L. McLellan
Date: 9-12-86

William Greear, Ph.D.
EPA Reviewer

Signature: William Greear
Date: 9/16/86

Albin Kocialski, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

A. Under the conditions of the assay, eight doses of AC 92,553 ranging from 50 to 5000 µg/plate caused a >2-fold dose-related increase in the number of revertants in Salmonella typhimurium strains TA1538 and TA98 (frame-shift mutation) with S9 activation. The positive controls demonstrated the sensitivity of the assay to detect a mutagenic response.

B. The study is acceptable.

Items 8 through 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix A for details.)

1. Test Material: AC 92,553, lot No. AC 3528-129-1, with a purity of 92.2% was not described. The test material was dissolved in dimethylsulfoxide (DMSO), the solvent control; all dose levels reported refer to the amount of active ingredient tested.

2. Tester Strains: The bacteria used in the assay were Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and Escherichia coli strain WP-2 uvrA$^-$. Salmonella strains were obtained from Dr. B. N. Ames, Berkeley, CA, and the Escherichia strain from Dr. B. Bridges, Brighton, England.

3. Positive Controls: The positive controls used were 2-nitrofluorene (2-NF), 9-aminoacridine (9-AA), 2-aminoanthracene (2-AA), N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and N-nitrosodimethylamine (DMNA).

4. S9 Fraction: S9 fractions were prepared from the livers of rats or hamsters that had been induced with Aroclor 1254.

5. Assay Methods:

a. Plate Incorporation: The appropriate test material dose, solvent or positive control, bacterial cells, and rat S9 mix or buffer were added to 2 mL of molten top agar containing trace amounts of histidine, biotin, and tryptophan. Tubes were mixed and poured onto minimal media, and plates were allowed to solidify. After a 48±6-hour incubation at 37°C, revertant colonies were counted.

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[1] Only items appropriate to this DER have been included.

To confirm phenotypes, revertant colonies were picked from selected dosed and solvent control plates and streaked onto fresh plates that were free of histidine. True revertants should be able to grow in the absence of added histidine. Plates were scored after a 24-hour incubation at 37°C.

b. Disc Test: The disc test was conducted similarly to the plate incorporation test, except the test material and solvent or positive controls were impregnated onto a paper disc as opposed to incorporation in the top agar.

c. Preincubation Assay: A 20-minute preincubation plate incorporation test with hamster S9 was used to assess the presence of mutagenic nitrosamine(s). The assay was performed with doses of the test material and solvent or positive controls using strain TA100. Preincubation was at room temperature. The methods used were described by Prival et al.[2]

6. Evaluation Criteria: In the plate incorporation assays the test material was considered positive (mutagenic) if the number of revertants at a given dose was greater than twice the number of revertants on the solvent control plates. The test material was considered positive in the disc test if it induced a ring of revertants around the disc.

B. Protocol: A protocol was not submitted.

12. REPORTED RESULTS:

A. Plate Incorporation Assay: In an initial assay, the test material was assayed at 50, 158, 500, 1581, and 5000 µg/plate with and without rat S9 activation. Precipitation of the test material was observed on all plates at 5000 µg/plate. The test material did not cause an appreciable increase (>2-fold) in the number of revertants compared to the solvent control with strains TA1535, TA1537, and WP-2 uvrA⁻ with or without S9 activation.

Slight increases with strain TA100 (1.6-fold increase for +S9 and a 1.4-fold increase for -S9) were observed at the highest dose, 5000 µg/plate. The test material with strains TA1538 and TA98 caused a 2.1-fold and <1-fold increase, respectively, at the highest dose without S9 activation. In the presence of S9 activation the test material caused a >2-fold dose-related increase in the number of revertants in both strain TA1538 and TA98 at all doses from 158 to 5000 µg/plate.

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[2] Prival, M. J., King, V., and Sheldon, A. The mutagenicity of dialkyl nitrosamines in the Salmonella plate assay. Environ. Mutat. 1(1979): 95-104.

To identify true revertants, a phenotype confirmation test was performed. Colonies were selected from plates with strain TA98, +S9, at test material doses of 500, 1581, and 5000 µg/plate and from plates with strains TA100 and TA1538, +/-S9, at dose levels of 1581 and 5000 µg/plate and were streaked onto fresh plates lacking histidine. The results indicated that the colonies were true revertants, i.e., all colonies grew in the absence of added histidine.

To confirm the responses noted in the initial assay, a partial repeat assay was performed with strains TA100 and TA1538 with and without S9 activation and with TA98 in the presence of S9 activation.

Nine test material doses ranging from 62.5 to 5000 µg/plate were assayed with strain TA98 in the presence of S9 activation. The test material precipitated at the three highest doses (2000, 3000, and 5000 µg/mL) and caused a >2-fold dose-related increase at dose levels >62.5 µg/plate. Similarly, 10 doses of the test material ranging from 31.25 to 5000 µg/plate with strain TA1538 in the presence of S9 activation precipitated at the two highest doses (2000 and 5000 µg/plate) and a dose-related, 2-fold increase in the number of revertants was observed. However, seven to nine doses of the test material ranging from 62.5 to 5000 µg/plate did not cause an increase in the number of revertants with strain TA1538, -S9, or TA100, +/-S9.

The minimal responses observed in the initial assay with strain TA100, +/-S9 and with TA1538, -S9, were not reproducible. However, the positive results for TA98 and TA1538 with S9 activation persisted. Therefore, another repeat assay was performed with strains TA98 and TA1538 at dose levels of 500, 750, 1000, 3000, and 5000 µg/plates. The test material caused, with increasing doses, a 2.3- to 4.4-fold increase in the number of revertants with strain TA98 and a 4.7- to 9.6-fold increase with strain TA1538. No precipitation was reported at these doses. The positive (mutagenic) results for both strains in the presence of rat S9 activation were confirmed.

The sensitivity of the initial and repeated assays to detect a mutagenic response was adequately demonstrated by the appropriate positive controls, MNNG, 9-AA, 2-NF, and 2-AA.

Representative results of the initial and two repeat assays are presented in Tables 1, 2, and 3.

B. Disc Test: The test material was assayed at 1000 µg/disc with and without S9 activation with S. typhimurium strains TA1535, TA1537, TA1538, TA98, and TA100 and E. coli strain WP-2 uvrA⁻. The test material did not induce a ring of revertants around the disc; therefore, the test material was considered nonmutagenic. The positive controls MNNG, 9-AA, 2-AA, and 2-NF induced positive responses.

TABLE 1. Results from the Initial Reverse Mutation Microbial Assay in S. typhimurium and E. coli with AC 92,553

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Substance (and Dose)		S9 Activation	Average Revertants[a]/Plate with Bacterial Strains TA1535	TA1537	WP-2 uvrA⁻	TA100	TA1538	TA98
Solvent Control								
DMSO		+	13	8	5	107	14	23
		–	18	7	7	115	15	21
Positive Controls[b]								
2-AA	5 µg/plate	+	284	436	—	2157	1570	1709
MNNG	10 µg/plate	+	—	—	585	—	—	—
		–	966	—	630	1241	—	—
9-AA	50 µg/plate	–	—	99	—	—	—	—
2-NF	20 µg/plate	–	—	—	—	—	1169	791
Test Material								
AC 92,553	50 µg/plate	+	9	8	5	134	24 (1.7)[c]	31 (1.3)
	158	+	17	8	7	141	57 (4.0)	51 (2.2)
	500	+	12	12	5	132	79 (5.6)	53 (2.3)
	1581	+	12	7	6	137	98 (7.0)	68 (3.0)
	5000[d]	+	15	12	5	167 (1.6)	122 (8.7)	106 (4.6)
	50 µg/plate	–	24	5	7	109	15	14
	158	–	18	5	7	104	14	17
	500	–	19	5	6	110	16	17
	1581	–	14	6	5	128	21	20
	5000[d]	–	15	8	5	165 (1.4)	32 (2.1)	20 (<1.0)

[a] Average Revertants = Averaged from triplicate plates.

[b] Positive controls: All controls caused a >2-fold increase in revertants.

[c] (N) = Fold increase over solvent control; calculated by reviewers.

[d] Precipitation observed at this dose on all plates.

TABLE 2. Representative Results from the First Repeat Reverse Mutation in S. typhimurium with AC 92,553

Substance (and Dose)		S9 Activation	Average Revertants[a]/ Plate with Bacterial Strains TA98	TA1538	TA100
Solvent Control					
DMSO		+	20	20	85
		–	--	15	91
Positive Controls					
2-AA	5 µg/plate	+	1324 (66.2)[b]	815 (40.8)	1199 (14.1)
2-NF	20 µg/plate	–	--	758 (50.5)	--
MNNG	10 µg/plate	–	--	--	357 (3.9)
Test Material					
AC 92,553	250 µg/plate	+	46 (2.3)	66 (3.3)	80 (<1.0)
	500	+	53 (2.7)	78 (3.9)	86 (1.0)
	1000	+	62 (3.1)	89 (4.5)	100 (1.2)
	5000[c]	+	100 (5.0)	175 (8.9)	111 (1.3)
	250	–	--	15 (1.0)	87 (<1.0)
	500	–	--	18 (1.2)	85 (<1.0)
	1000	–	--	23 (1.5)	95 (1.0)
	5000[c]	–	--	28 (1.9)	99 (1.1)

[a] Average Revertants = Averaged from triplicate plates.

[b] (N) = Fold increase over solvent control; calculated by reviewers.

[c] Highest precipitated dose.

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TABLE 3. Results from the Second Repeat Reverse Mutation Assay in S. typhimurium with AC 92,553

Substance (and Dose)	S9 Activation	Average Revertants[a]/ Plate with Bacteria Strains TA98	TA1538
Solvent Control			
DMSO	+	25	15
Positive Control			
2-AA 5 µg/plate	+	1222 (48.9)[b]	931 (62.1)
Test Material			
AC 92,553 500 µg/plate	+	58 (2.3)	71 (5.0)
750	+	61 (2.4)	84 (5.6)
1000	+	62 (2.5)	102 (6.8)
3000	+	93 (3.7)	129 (8.6)
5000	+	109 (4.4)	145 (9.7)

[a]Average Revertants = Averaged from triplicate plates.

[b](N) = Fold increase over solvent control; calculated by reviewers.

C. Preincubation Assays: To assess the presence of mutagenic nitrosamine(s), test material doses of 1000, 5000, 7500, and 10,000 µg/plate were incubated with hamster S9 activation for 20 minutes at room temperature. No precipitation of the test material was reported at doses tested. The test material did not cause an appreciable increase in the number of revertants when compared to the solvent control. The positive control, DMNA, caused an increase (3.3-fold) in the number of revertants, demonstrating the sensitivity of the assay to detect mutagenic nitrosamine. Representative results of the preincubation assay are presented in Table 4.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. The study authors concluded that "In summary, AC 92,553 Lot AC 3528-129-1 showed mutagenic activity with strains TA 98 and TA 1538 in the presence of aroclor-1254 induced rat liver S-9. No activity was found with strains TA 1535, TA 1537, TA 100 or WP-2 uvrA$^-$. In addition, no activity was found with strain TA 100 using the preincubation procedure with hamster S-9 to detect mutagenic nitrosamine activity."

B. A quality assurance statement was signed and dated October 24, 1985.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

We assess that the study authors correctly interpreted the data and that AC 92,553 caused frame-shift mutations in S. typhimurium strains TA98 and TA1538 with rat S9 activation in the plate incorporation assay. The positive controls adequately demonstrated the sensitivity of the assay to detect a mutagenic effect.

The negative result observed in the disc test was inconclusive because the assay was performed with only one dose and in single plates; therefore, this assay did not add any usable information to the overall assessment of the test material.

The study authors performed a preincubation assay with strain TA100 in the presence of hamster S9 activation to detect mutagenic activity due to the presence of nitrosamines. The study authors concluded that the test material was not mutagenic. However, we assess that the study authors should have included strains TA1538 and TA98 since a mutagenic response had been observed with both strains using rat S9 activation; hence, the assay should have been performed also with and without hamster S9 activation.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 5-13.

TABLE 4. Representative Results from the Preincubation Reverse Mutation Assay in S. typhimurium with Hamster S9 Activation and AC 92,558

Substance (and Dose)	S9 Activation	Average Revertants[a]/Plate TA100
Solvent Control		
DMSO	+	121
Positive Control		
DMNA 1230 µg/plate	+	395 (3.3)[b]
Test Material		
AC 92,553 1000 µg/plate[c]	+	145 (1.2)
10,000 µg/plate[d]	+	159 (1.3)

[a]Average Revertants = Averaged from triplicate plates.

[b](N) = Fold increase over solvent control; calculated by reviewers.

[c]Lowest dose tested.

[d]Highest dose tested.

APPENDIX A

Materials and Methods

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