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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Cyanazine; Reregistration Case #0066; ID #100101; Review of New Toxicology Studies Submitted in Support of Reregistration

Tox.Chem No.: 188C
MRID No.: 427395-01, -02, -03, -04
DP Barcode No.: D190858
Submission No.: S440035

TO: Walter Waldrop/Venus Eagle, PM #71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

FROM: William Dykstra, Ph.D., Toxicologist
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)
William Dykstra 6/22/93

THRU: Roger Gardner, Section Head, Toxicologist
Review Section I
Toxicology Branch I
Health Effects Division (H7509C)
Roger Gardner 6-24-93
KB 7/2/93

ACTION REQUESTED: As part of the FIFRA '88 Reregistration Process, the Registrant, E. I. du Pont de Nemours and Company, has submitted four studies to satisfy the Guideline Requirements: 81-4 (Primary Eye Irritation Study in Rabbits), 81-5 (Primary Dermal Irritation Study in Rabbits), 81-6 (Dermal Sensitization Study in Guinea Pigs) and 84-2 (Mutagenicity Study: Other Genotoxic Effects).

Toxicology Branch-I (TB-I) has been requested to review these studies and determine if they fulfill the Guideline Requirements.

CONCLUSIONS: Each of the four studies is acceptable and fulfills the Guideline Data Requirement for 81-4, 81-5, 81-6, and 84-2. Summaries of each of the studies are presented below:

81-4 Cyanazine technical was tested in the right eye of each of 6 NZW rabbits, with the left eye serving as a control. Observations were at 1, 24, 48, and 72 hours after instillation of 59 mg of technical cyanazine without washing.

Cyanazine technical produced iritis and conjunctival redness, chemosis, and discharge, but no corneal opacity. Since there was no corneal involvement and all irritation cleared in 7 days or less (at 72 hours, all scores were zero), the correct classification is **Toxicity Category III.**

81-5 Cyanazine technical was tested according to recommended Agency procedures and had a primary irritation index of 0.6, which is classified as mildly or slightly irritating, **Toxicity Category IV.**

81-6 Cyanazine technical was tested for dermal sensitization using the Modified Buehler Method in 20 male guinea pigs. Cyanazine technical was negative for dermal irritation during both the induction and challenge phases of the study when tested as 0.4 grams in 0.4 ml of saline (concentration was based on a pilot study). Therefore, cyanazine technical is not a dermal sensitizer. The positive control, DNCB, produced a dermal sensitization reaction in each of 5 positive control guinea pigs.

84-2 Cyanazine technical was tested for its ability to induce unscheduled DNA synthesis (UDS) in spermatocytes of young adult male Sprague-Dawley rats. Groups of rats were exposed by oral gavage to 0, 125, 185, 250, and 500 mg/kg each day for five consecutive days. Positive contols were MMS and CP. All cyanazine treated animals lost statistically significant amounts of body weight. Some rats in the 500, 250, and 185 mg/kg groups had clinical signs and deaths were reported in those groups. Cyanazine was tested at appropriately high doses. Testicular cells were prepared for UDS evaluation at approximately 2 and 24 hours after the last dose. Increased UDS was not observed at any dose at any harvest time. Based on these results, cyanazine technical is negative for UDS

activity in this assay.

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Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 5/18/93
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I Roger Gardner 6-24-93

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DATA EVALUATION REPORT

STUDY TYPE: 81-4; Primary Eye Irritation Study with DPX-R1957-75 (Cyanazine) in Rabbits

TOX. CHEM NO: 188C

MRID NO.: 427395-01

TEST MATERIAL: Cyanazine technical

SYNONYMS: Bladex

STUDY NUMBER: HLR No. 57-93; Medical Research No. 9581-034

SPONSOR: E. I. du Pont de Nemours and Co.

TESTING FACILITY: Haskell Laboratry for Toxicology

TITLE OF REPORT: Primary Eye Irritation Study with DPX-R1957-75 (Cyanazine) in Rabbits

AUTHOR(S): John W. Sarver

REPORT ISSUED: March 31, 1993

CONCLUSION: Cyanazine technical was tested in the right eye of each of 6 NZW rabbits, with the left eye serving as a control. Observations were at 1, 24, 48, and 72 hours after instillation of 59 mg of technical cyanazine without washing.

Cyanazine technical produced iritis and conjunctival redness, chemosis, and discharge, but no corneal opacity. Since there was no corneal involvement and all irritation cleared in 7 days or less (at 72 hours, all scores were zero), the corrct classification is **Toxicity Category III.**

Core Classification: **ACCEPTABLE**

1. Quality Assurance Statement: A Quality Assurance Documentation was present, signed by Linda S. Mauzy, and dated March 24, 1993.

2. Test Material: Cyanazine technical, DPX-R1957-75, cream solid, Lot No. 00048315, 98.6% purity, was used as the test material.

3. Animals: 6 NZW rabbits used in the study were purchased from Hare Marland, Hewitt, New Jersey. The rabbits were individually caged and fed approximately 125 grams of Purina Certified High Fiber Rabbit Chow™ #5325 daily during the study. Tap water was available ad libitum.

4. Methods: On the day prior to treatment, the eyes of 1 male and 5 female NZW rabbits were examined using illumination, magnification, and fluorescein stain. Only animals free of pre-existing injury were used in the study. The rabbits weighed from 3008 to 3576 grams on the day of treatment.

Approximately 59 mg of test material (a weight equivalent to a 0.1 ml volume of test material) was placed into the lower conjunctival sac of the right eyes of 6 NZW rabbits. The left eye served as a control and was left untreated. The treated and control eyes were left unwashed. At 1, 24, 48, and 72 hours after administration, the rabbits were examined for evidence of eye irrritation according to the Draize system of scoring using illumination and magnification. Hemastix™ reagent strips were used to detect occult blood in the discharge from the eyes. Control eyes were not scored and were considered normal relative to the treated eyes.

RESULTS

Incidences of Positive Ocular Responses

Response	1 hr	24 hr	48 hr	72 hr
Corneal Opacity	0/6	0/6	0/6	0/6
Iritis	4/6	1/6	0/6	0/6
Conjunctiva				
Redness	6/6	2/6	2/6	0/6
Chemosis	0/6	1/6	0/6	0/6

Mean Scores for Ocular Responses

	Mean Readings At 24 hr	48 hr	72 hr	Average
Conjunctiva				
Chemosis	1.0	0.33	0.0	0.44
Redness	1.33	1.33	0.0	0.89
Iritis	0.17	0.0	0.0	0.06
Corneal Opacity	0.0	0.0	0.0	0.0

CONCLUSION:

Cyanazine technical was tested in the right eye of each of 6 NZW rabbits, with the left eye serving as a control. Observations were at 1, 24, 48, and 72 hours after instillation of 59 mg of technical cyanazine without washing.

Cyanazine technical produced iritis and conjunctival redness, chemosis, and discharge, but no corneal opacity. Since there was no corneal involvement and all irritation cleared in 7 days or less (at 72 hours, all scores were zero), the corrct classification is **Toxicity Category III.**

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 5/27/93
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I Roger Gardner 6-24-93

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DATA EVALUATION REPORT

STUDY TYPE: 81-5; Primary Dermal Irritation Study with DPX-R1957-75 (Cyanazine) in Rabbits

TOX. CHEM NO: 188C

MRID NO.: 427395-02

TEST MATERIAL: Cyanazine technical; 98.6% purity

SYNONYMS: Bladex

STUDY NUMBER: HLR No. 34-93, Medical Research No. 9581-034

SPONSOR: E. I. du Pont de Nemours and Co., Newark, Delaware

TESTING FACILITY: Haskell Laboratory for Toxicology

TITLE OF REPORT: Primry Dermal Irritation Study with DPX-R1957-75 (Cyanazine) in Rabbits

AUTHOR(S): John W. Sarver

REPORT ISSUED: March 31, 1993

CONCLUSION: Cyanazine technical was tested according to recommended Agency procedures and had a primary irritation index of 0.6, which is classified as mildly or slightly irritating, **Toxicity Category IV.**

Core Classification: **ACCEPTABLE**

1. Quality Assurance Documentation: A signed statement of Quality Assurance inspections and reports of findings was present, signed by Kimberly B. Brebner and dated March 30, 1993.

2. Test Material: DPX-R1957-75 (Cyanazine), cream solid, Lot No. 00048315, 98.6% purity, was the test material used in the study.

3. Animals: 6 NZW rabbits, purchased from Hare Marland, Hewitt, New Jersey, were used in the study. The animals were individually caged and fed Purina Certified High Fiber Rabbit Chow™ #5325 and tap water ad libitum. On the day of treatment, the hair was closely shaved to expose the skin from the scapular to the lumbar region of the back. The rabbits weighed from 2708 to 3210 grams on the day of treatment.

4. Methods: Approximately 0.5 grams of test material, moistened with deionized water, was applied to a 2-inch square gauze pad that was placed on the intact skin. The patch was held in place with non-irritating tape. The test sites were then wrapped with rubber sheeting and secured in place for 4 hours. The test sites were evaluated for erythema, edema, and other evidence of dermal effects approximately 1 hour after removal of the patches and were scored according to the Draize score. Additional evaluations were made at 24, 48, and 72 hours after removal of the patches.

Results:

Individual scores for each animal during the study are presented below:

Erythema

Rabbit Number	1 hr	24 hr	48 hr	72 hr	day 6	day 7	day 8
27703	0	0	0	0	-	-	-
27704	0	0	0	0	-	-	-
27705	0	1	0	0	-	-	-
27706	0	2	2	2	2S,L	1S,L	0
27707	0	2	2	2	1S,L	0S,L	0
27709	0	0	0	0	-	-	-

Edema

Rabbit Number	1 hr	24 hr	48 hr	72 hr	day 6	day 7	day 8
27703	0	0	0	0	-	-	-
27704	0	0	0	0	-	-	-
27705	0	0	0	0	-	-	-
27706	0	1	0	0	0	0	0
27707	0	1	0	0	0	0	0
27709	0	0	0	0	-	-	-

S = Epidermal Scaling
L = Sloughing

Primary Irritation Index

Rabbit Number	Primary Dermal Irritation Scores
27703	0.0
27704	0.0
27705	0.2
27706	1.8
27707	1.8
27709	0.0

Primary Irritation Index = 0.6

Conclusion: Cyanazine technical was tested according to recommended Agency procedures and had a primary irritation index of 0.6, which is classified as mildly or slightly irritating, **Toxicity Category IV.**

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 5/20/93
Review Section I, Tox. Branch I
Secondary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I Roger Gardner 6-24-93

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DATA EVALUATION REPORT

STUDY TYPE: 81-6; Dermal Sensitization Study-Guinea Pig

TOX. CHEM NO: 188C

MRID NO.: 427395-03

TEST MATERIAL: Cyanazine Technical, 98.6% purity, DPX-R1957-75

SYNONYMS: Bladex

STUDY NUMBER: HLR No. 846-92; Medical Research No. 9581-034

SPONSOR: E. I. du Pont de Nemours and Company

TESTING FACILITY: Biosearch Incorporated, Philadelphia, PA, for Haskell Laboratory for Toxicology

TITLE OF REPORT: Delayed Contact Hypersensitivity Test (Buehler Method) with DPX-R1957-75 (Cyanazine) in Guinea Pigs

AUTHOR(S): Pamela Romanelli

REPORT ISSUED: February 2, 1993

CONCLUSION: Cyanazine technical was tested for dermal sensitization using the Modified Buehler Method in 20 male guinea pigs. Cyanazine technical was negative for dermal irritation during both the induction and challenge phases of the study when tested as 0.4 grams in 0.4 ml of saline (concentration was based on a pilot study). Therefore, cyanazine technical is not a dermal sensitizer. The positive control, DNCB, produced a dermal sensitization reaction in each of 5 positive control guinea pigs.

Core Classification: **ACCEPTABLE**

1. Quality Assurance Statement: A Quality Assurance Statement was present, signed by Paul J. Briddock. Quality Assurance Coordinator, and Dr. James E. Long, Director, Quality Assurance and dated January 28, 1993.

2. Test Material: Cyanazine Technical, DPX-R1957-75, purity 98.6%, Lot No. 00048315, cream solid

   Positive Control: DNCB, 99+%, 1-chloro-2,4-dinitrobenzene, Kodack Laboratory Chemicals, Lot #A11R

3. Animals: Male Hartley guinea pigs, 326 to 409 grams, were used in the study The animals were purchased from Davidson Mill Farm, Jamesburg, NJ and individually caged. Guinea pig formula and tap water were available ad libitum. Animals were acclimated for 7 days prior to initiation of the study.

4. Range Finding Study: The irritation potential of technical cyanazine was determined. In this test, both sides of 4 animals were closely clipped and exposed to 0.4 g and 0.2 g of test material, moistened with an equal amount of saline. No dermal irritation was observed at either concentration; therefore, the induction and challenge applications were performed using 0.4 g of the test material, moistened with 0.4 ml of saline. Screen animal #1 had a prolapsed rectum and was euthanized.

5. Methods: **INDUCTION**

   Test Material Group

   One day prior to study initiation and as often as needed, a group of 20 guinea pigs was closely clipped over the induction site on the left flank. A 0.4 g of test material, moistened with 0.4 ml saline, was applied to a gauze patch (1 x 1 inch) which had been placed onto a piece of plastic film. The patch was placed onto the prepared skin test site and the plastic material was wrapped snugly around the trunk of the animal and secured with an elastic tape. The patch was removed after 6 hours, the test site wiped with deionized water and the test site was scored for irritation at 24 and 48 hours from initial patch application. This procedure was performed once a week for three weeks, for a total of three, six hour applications with test material.

Vehicle Control Group

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A group of 10 guinea pigs was treated in the same manner as the test material group, except that a 0.4 ml portion of saline was applied for 6 hours instead of test material. This procedure was performed once a week for three weeks, for a total of three, six-hour applications with the vehicle.

Positive Control Group

A group of 5 guinea pigs was treated in the same manner as the test material group, except that a 0.4 ml portion of 0.1% DNCB w/v in 50% ethanol: 0.9% saline was applied for 6 hours instead of test material. This procedure was performed once a week for three weeks, for a total of three, six-hour applications with the positive control material.

CHALLENGE

Test Material Group

After the third induction application, the animals were rested for 14 days. After the rest period, a challenge application, using the methods previously described, was applied to a naive challenge site (on the clipped right flank) for 6 hours. After removal of the challenge application, the site was examined for dermal irritation and/or signs of sensitization at 24 and 48 hours after the challenge application.

Vehicle Control Group

After the third induction application, the animals were rested for 14 days. After the rest period, the vehicle control group was challenged with the vehicle on the clipped left flank (caudal) and the test material on the right flank, using the methods previously described for 6 hours. After removal of the challenge application, the sites were examined for dermal irritation and/or signs of sensitization at 24 and 48 hours after the challenge applications.

Positive Control Group

After the third induction application, the animals were rested for 14 days. After the rest period, a challenge application, using the methods previously described, was applied to a naive challenge site (on the clipped right flank) for 6 hours. A group of 5 naive animals was also treated at the time of challenge. After removal of the challenge application, the site was examined for dermal irritation and/or signs of sensitization at 24 and 48 hours after the challenge application.

Scale for Evaluation of Dermal Irritation

0 = no reaction

+ = slightly patchy redness

1 = slight or confluent moderately patchy redness or area

2 = moderate redness

3 = severe redness with/without swelling

RESULTS

INDUCTION and CHALLENGE

Test Material Group

Following the initial induction application, hyperactivity and ruffled fur were observed on the following day. Ruffled fur was observed for 5 days after each induction application and for 2 days after the challenge application. Animal #30 had a prolapsed rectum and colon and was euthanaized on Day 14. Animals gained weight during the study. There was no dermal irritation after the induction applications or challenge application in any animal. Therefore, the test material, cyanazine technical, was not a sensitizer.

Vehicle Control Group

All animals appeared normal during the induction phase, but after challenge, they had ruffled fur. Animals gained weight during the study. There was no dermal irritation after any of the induction applications. Additionally, there was no dermal irritation for any of the animals after the challenge applications of the test material (right side) and vehicle (left side).

Positive Control Group

All animals appeared normal and gained weight during the study. No dermal irritation to moderate redness (grades of 0 to 2) was observed during the induction phase of the study. After the challenge application, the incidence of sensitization was 5/5 and severity was 1.8 (four animals had 2 and one animal had 1) at 24 hours and 0.8 (one with 2, one with 0, one with 1, and two with +) at 48 hours. All naive positive control animals had 0 scores for dermal irritation. It was concluded that the positive control was a dermal sensitizer in this study.

CONCLUSIONS

Cyanazine technical was tested for dermal sensitization using the Modified Buehler Method in 20 male guinea pigs. Cyanazine technical was negative for dermal irritation during both the induction and challenge phases of the study when tested as 0.4 grams in 0.4 ml of saline (concentration was based on a pilot study). Therefore, cyanazine technical is not a dermal sensitizer. The positive control, DNCB, produced a dermal sensitization reaction in each of 5 positive control guinea pigs.

Reviewed by: William Dykstra, Ph.D. Toxicologist William Dykstra 6/22/93
Review Section I, Tox. Branch I
Secondary Reviewer: Kerry Dearfield, Ph.D., Geneticist Kerry Dearfield
Section Head, Science Analysis Branch Roger Gardner
Tertiary Reviewer: Roger Gardner, Section Head
Review Section I, Tox Branch I 6-24-93

010382

DATA EVALUATION REPORT

STUDY TYPE: 84-2; Other Genotoxic Effects; UDS in Rat Spermatocytes (Determination of Genetic Damage)

TOX. CHEM NO: 188C

MRID NO.: 427[illegible]-04

TEST MATERIAL: Cyanazine Technical; DPX-R1957-75

SYNONYMS: Bladex

STUDY NUMBER: Haskell Lab Report No. HRL 281-93

SPONSOR: E. I. du Pont de Nemours and Company, Newark, DE

TESTING FACILITY: Haskell Laboratory for Toxicology and Industrial Medicine

TITLE OF REPORT: Determination of Unscheduled DNA Synthesis in Rat Spermatocytes Following In Vivo Exposure to DPX-R1957-75 (Cyanazine) by Oral Gavage

AUTHOR(S): Karin S. Bentley

REPORT ISSUED: April 8, 1993

CONCLUSION: Cyanazine technical was tested for its ability to induce unscheduled DNA synthesis (UDS) in spermatocytes of young adult male Sprague-Dawley rats. Groups of rats were exposed by oral gavage to 0, 125, 185, 250, and 500 mg/kg each day for five consecutive days. Positive contols were MMS and CP. All cyanazine treated animals lost statistically significant amounts of body weight. Some rats in the 500, 250, and 185 mg/kg groups had clinical signs and deaths were reported in those groups. Cyanazine was tested at appropriately high doses. Testicular cells were prepared for UDS evaluation at approximately 2 and 24 hours after the last dose Increased UDS was not observed at any

dose at any harvest time. Based on these results, cyanazine technical is negative for UDS activity in this assay.

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Core Classification: **ACCEPTABLE**

Quality Assurance Statement: Quality Assurance Documentation was present, signed by Robert C. Rhea, Quality Assurance Auditor, and dated April 4, 1993

Test Material: Cyanazine technical, DPX-R1957-75 (R1957-75), Lot No. 00048315, 98.6% purity, cream solid

Negative Control: the vehicle, 0.5% methyl cellulose, was the negative control for all treatments.

Positive Control: Two positive controls were given separately to different groups of animals. Methyl methanesulfonate (MMS), a direct acting mutagen, was given at a dose of 50 mg/kg body weight. Cyclophosphamide (CP), a mutagen requiring metabolic activation, was given at a dose of 100 mg/kg. The two positive controls were given by intraperitoneal (IP) injection.

Animals: Sixty-five male Sprague-Dawley rats (57 days of age) were purchased from Charles River (Kingston,NY). The animals were individually caged and fed Purina Rodent Chow #5002 (Lot No. AU26922B) and tap water ad libitum. At the time the study was conducted, the rats were 9 weeks old. The mean pre-treatment weight of the animals evaluated for UDS was 322.3 grams (range 290.8-349.9 grams) with the exception of six rats which did not begin to be dosed until Day 4 of the study (see Results). The mean pre-treatment weight of those six rats was 364.8 grams (range 319.4-386.2 grams). Rats were randomly assigned to control and treatment groups in such a manner that the pretest mean body weights were not statistically different ($p > 0.05$).

Dose Selection: In a rangefinding experiment, groups of 5 male rats were dosed orally with 100, 200, 300, 400, and 500 mg/kg of Cyanazine technical on 5 consecutive days (survival permitting). Animals were weighed and observed daily during the 5 day period and 24

hours following the last treatment.

Clinical signs included lethargy, diarrhea, piloerection, and a red-to-brown discharge around the eyes. Those signs were seen more frequently in rats receiving 300, 400, and 500 mg/kg. On Day 3, one rat in each of the 200 and 300 mg/kg groups was found dead, and on day 4, one rat dosed at 500 mg/kg was found dead. No marked signs of toxicity were seen in animals given 100 and 200 mg/kg. Based on this information, the doses selected for the UDS study were 125, 250, and 500 mg/kg. Due to excessive mortality at 500 mg/kg during the main study, however, an additional dose of 185 mg/kg was included.

Compound Preparation:

A. Test Material: The test material, cyanazine technical, was prepared in 0.5% methyl cellulose at dosing concentrations of 0 (negative control), 25, 37, 50, and 100 mg/ml immediately prior to each dosing day. The treatments were administered by oral intubation at a 5 ml/kg volume, yielding effective doses of 0, 125, 185, 250, and 500 mg/kg. Dosing solutions were not analyzed for concentration, uniformity, or stability.

B. Positive Controls:

MMS was prepared in phosphate buffered saline at a concentration of 25 mg/ml. MMS was administered at a volume of 2 ml/kg, yielding an effective dose of 50 mg/kg.

CP was prepared in 0.9% sterile saline at a concentration of 50 mg/ml. CP was administered at a volume of 2 ml/kg, yielding an effective dose of 100 mg/kg.

Treatment Schedule:

Cyanazine technical was administered orally, by gavage, at doses of 0, 125, 250, and 500 mg/kg B.W. for 5 consecutive days to groups of 10 to 20 male rats. One group of 5 animals were concurrently treated with 100 mg/kg CP for the same 5 day period. Animals treated with MMS were given a single i.p.

injection 2 hours prior to scheduled sacrifice on Day 5 of the study. Due to excessive mortality at 500 mg/kg, an additional group of animals was given, by gavage, 185 mg/kg of cyanazine technical. This group consisted of 2 rats originally designated for treatment with MMS as well as 5 unassigned rats which were received in the original shipment. Dosing began on Day 4 of the study and continued for 4 consecutive days.

Groups of 3 to 5 rats were sacrificed at approximately 2 and 24 hours after administration of cyanazine technical at 0, 125, 185, and 250 mg/kg. All remaining rats in the 500 mg/kg group and the positive control rats were sacrificed 2 hours post-treatment. All rats were observed daily for clinical signs and body weights were taken prior to dosing each day. At the appropriate time, rats were sacrificed by CO_2 asphyxiation and one testis from each animal was removed.

Preparation and Culture of Testicular Cells:

Immediately following sacrifice, each testis was placed in a sterile dish containing an enriched Krebs/Ringer bicarbonate solution (EKRB). The testes were decapsulated and the seminiferous tubules were washed in EKRB. Tubules were enzymatically digested in two steps in a solution of EKRB containing, first, type I collagenase followed by trypsin and type II deoxyribonuclease I in EKRB. Tubules were then gently drawn up and down in a pipet to produce a cell suspension. Large tubule fragments were allowed to settle and the supernatant was centrifuged at approximately 1000 rpm. Cell pellets were washed and resuspended in EKRB. Viability (exclusion of trypan blue) and cell density were determined. Individual wells of a 24-well culture plate containing 1 ml of medium were inoculated with 6×10^5 viable testicular cells. Cells were cultured in Williams E medium supplemented with 2% fetal bovine serum, and approximately 10 μCi/ml [methyl-H^3] thymidine. Cultures were incubated for 22 to 24 hours in a humidified $5 \pm 1\%$ CO_2 atmosphere at $33 \pm 1°C$.

Fixation and Autoradiography:

Following incubation, cell viability was determined by trypan blue. Cells were fixed in 10% formaldehyde, washed with PBS and aliquots of each suspension were placed on microscope slides. Slides were coded and identified with the last three digits of the animal number. Slides were washed in cold water, methanol, and when dry, dipped into undiluted Kodak™ nuclear track emulsion and stored frozen for three weeks to expose the emulsion. Slides were then developed and stained with Gills hematoxylin.

Scoring of UDS:

Slides were randomized and coded to assure unbiased scoring. UDS was evaluated in mid- to late-stage pachytene spermatocytes which were identified by the criteria of Leblond and Clermont (1952). Silver grains were counted under a 100x oil immersion light microscope objective with a 10x eye piece. Counts were made using an Artek™ colony counter interfaced via a remote Artek™ TV camera to a microscope. Twenty-five cells from each slide, three slides per animal, were scored for a total of 75 cells per animal. For each cell, the area of the sliver grains over the nucleus was measured. As pachytene spermatocytes contain little cytoplasm, traditional cytoplasmic measurements could not be made. However, areas of grains in two or more nuclear sized regions adjacent to the nucleus were measured to provide background grain counts. The highest background value was recorded.

Data Calculations and Statistical Analyses:

Background grain counts were subtracted from the nuclear grain counts to determine the net nuclear grains (NNG) of each cell. The mean NNG and standard error of the mean (SEM) were calculated for each slide. Mean nuclear grains, mean background grains, and median NNG were also calculated. The mean NNG per cell and the SEM were then calculated for each animal using the average slide values. The percent of cells in repair (percent of cells having more NNG than 95% of control

cells) was also calculated.

Statistical analyses were done using the animal as the experimental unit. The mean NNG per cell for cyanazine exposed animals was compared to the negative controls at each harvest time. Nonparametric methods using the Kruskal-Wallis test were employed for the NNG data since the data were found not to be normally distributed. Weight change data were analyzed using an analysis of variance, and aposteriori comparisons to control were made using Dunnett's test. The positive controls were not included in the analysis of cyanazine. These analyses were conducted at a 5% level of significance. NNG counts for cyanazine treated animals were also analyzed for dose-related trends using the Jonckheere-Terpstra trend test at a 1% level of significance.

Acceptability and Classification Criteria:

A. Acceptability

1. A slide was rejected if there were fewer than 25 scorable slides.
2. Slides for an animal were rejected if there were fewer than 75 scorable slides.
3. The study was rejected if the mean NNG per cell for the positive controls was not significantly greater than that for the negative controls.

B. Classification

The test material is considered **POSITIVE** if:
1. The mean NNG per cell for a group of animals exposed to the test material is statistically greater than the mean NNG per cell for the corresponding group of negative control animals ($p \leq 0.05$)

AND

2. The average percent of cells undergoing repair for those animals is 10% or greater.

The test material is considered **NEGATIVE** if:
1. The mean NNG per cell for all groups of animals exposed to the test material is not statistically greater than that for the

corresponding negative control animals ($p > 0.05$).

AND

2. The average percent of cells undergoing repair is less than 10%.

RESULTS

1. Clinical Findings: After one hour, the 20 animals receiving the first dose of the 500 mg/kg were lethargic, had diarrhea by Day 2, and at which time 1 animal was found dead. An additional 9 rats died by Day 3 and within 4 hours of dosing on Day 3, 5 more rats died in this group. On Day 4, another rat was found dead and the 4 remaining rats were lethargic and 1 rat had diarrhea.

At 250 mg/kg, 1 rat out of ten died by the 3rd study Day; another was dead on Day 4. Transient signs of toxicity seen in survivors were diarrhea, piloerection, and lethargy.

No clinical signs were seen in the 125 mg/kg dose group, and the negative and positive controls.

Due to excessive mortality in the 500 mg/kg group, an additional group of rats was gavaged with 185 mg/kg cyanazine technical (this would allow for 3 doses to assess any dose response if one occurred). This group consisted of 2 rats originally designated for treatment with MMS and 5 unassigned rats which were part of the original shipment. Dosing began on Day 4 of the study and continued an additional 4 consecutive days. Clinical signs included diarrhea, lethargy, and red-to-brown staining of the underbody, nose, and mouth. One rat from this group died on the 5th day of dosing.

2. Body Weight Effects: Body weight losses were observed in all groups of animals exposed to cyanazine technical at all doses and all harvest times. The body weight

losses were statistically significant in comparison to negative controls.

3. UDS Findings: Cyanazine technical was not toxic to rat testicular cells. Following isolation, testicular-cell viability ranged from 96.5 to 99.2% for negative control animals and 90.1 to 99.1% for rats exposed to cyanazine technical. There were no statistically significant increases or dose-related increased trends in UDS response observed at any dose of cyanazine technical at either harvest time. The positive controls produced statistically significant increases in NNG. The UDS data are summarized below.

UDS SUMMARY

Dose (mg/kg)[a]	Harvest Time (hrs)[b]	Number of Animals	Mean Net Nuclear Grains/Cell ± SEM[c]	Mean % Cells Responding ± SEM
0	2	5	7.3 ± 0.8	7.2 ± 3.1
	24	5	5.8 ± 0.6	6.1 ± 2.3
125	2	5	8.0 ± 1.1	8.8 ± 3.0
	24	5	5.4 ± 0.4	4.3 ± 1.1
185	2	3[c]	7.2 ± 1.0	7.6 ± 3.6
	24	3	5.4 ± 0.4	2.7 ± 2.1
250	2	4[c]	6.6 ± 1.0	5.7 ± 2.5
	24	4[c]	5.6 ± 0.3	4.0 ± 1.0
500	2	4[cd]	6.2 ± 1.1	7.0 ± 2.1
MMS (50 mg/kg)[e]	2	3[f]	29.2 ± 1.3*	85.8 ± 1.9
CP (100 mg/kg)	2	5	20.6 ± 1.1*	53.3 ± 4.7

a Administered five consecutive days.
b Time following last dose.
c Animal(s) died prior to scheduled sacrifice.
d Animal moved from the 24 to 2 hour sacrifice.
e Administered as a single i.p. injection on the fifth day of the study.
f Two animals moved to the 185 mg/kg cyanazine group.

Conclusion:

Cyanazine technical was tested for its ability to

induce unscheduled DNA synthesis (UDS) in spermatocytes of young adult male Sprague-Dawley rats. Groups of rats were exposed by oral gavage to 0, 125, 185, 250, and 500 mg/kg each day for five consecutive days. Positive controls were MMS and CP. All cyanazine treated animals lost statistically significant amounts of body weight. Some rats in the 500, 250, and 185 mg/kg groups had clinical signs and deaths were reported in those groups. Cyanazine was tested at appropriately high enough doses. Testicular cells were prepared for UDS evaluation at approximately 2 and 24 hours after the last dose. Increased UDS was not observed at any dose at any harvest time. Based on these results, cyanazine technical is negative for UDS activity in this assay.