CASWELL FILE
1-20-87

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

005681

JAN 20 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP#5G3283 - Experimental Use Permit (54555-EUP-R)
Request for the Use of Dormex™ on Grapes

485A
Tox. Chem. No. ~~266B~~
Acc. No. 073726
073727

FROM: William B. Greear, M.P.H. William B. Greear 12/31/86
Section VII, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Richard F. Mountfort, PM 23
Fungicide-Herbicide Branch
Registration Division (TS-767C)

THRU: Albin B. Kocialski, Ph.D., Supervisory Pharmacologist
Section VII, Toxicology Branch 1/5/87
Hazard Evaluation Division

ABK

and

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

S.R. Siemer of Siemer & Associates, Inc., has submitted a petition on behalf of SKW Trostberg AG requesting an Experimental Use Permit (54555-EUP-R) and a temporary exemption from the requirements of tolerance for the use of Dormex (hydrogen cyanamide) on grapes.

In a conversation with R. Mountfort on June 23, 1986, it was decided that because the application has been withdrawn, this memorandum should only address the adequacy of the sub-

312 1

5H 014002

mitted toxicology data. Numerous studies have been submitted for evaluation. The Data Evaluation Records are attached. The results of the studies are briefly summarized below and have been classified according to the "CORE" classification system. Toxicity categories have been assigned where required.

Study	Results/Toxicity Category	Classification
Acute Oral LD_{50}	II	Minimum
Acute Dermal LD_{50}	III	Supplementary
Acute Inhalation LC_{50}	III	Minimum
Primary Dermal Irritation	I	Minimum
Primary Dermal Irritation	IV	Invalid
Primary Eye Irritation	II	Minimum
Skin Sensitization*	Produces sensitization	Guideline (tentative)
28-Day Feeding (rat)	NOEL < 100 ppm	Supplementary
90-Day Feeding (rat)	NOEL = 20 ppm	Supplementary
Teratology (rabbit)	NOEL (developmental) = 12 mg/kg	Guideline
Oncogenicity (rat)**	Negative	Supplementary
Oncogenicity (mouse)**	Negative	Supplementary
Ames' Test**	Inconclusive	Unacceptable
Micronucleus Test*	Inconclusive	Unacceptable
Sister Chromatid Exchange*	Inconclusive	Unacceptable

It was noted that a report of the subchronic oral dog study was not included in the submission. Therefore, the sponsor should submit this study for evaluation.

There was much confusion concerning the test material used in the various submitted studies as well as how the test materials related to the actual product to be marketed. This information must be submitted as well as an updated Confidential Statement of Formula.

The protocol for the study entitled "Protocol for an Oral Two-Generation Reproduction Study with an Aqueous Cyanamide Solution (Content 49%) in Rats" was evaluated. The following changes are suggested:

1. Pups should be weighed on days 1, 4, 7, 14, and 21. Individual pup weights should be determined on day 21.

2. All tissues of parental animals with gross lesions should be examined microscopically.

3. All pups should have a gross necropsy.

Attachment

* The composition of the test material is unknown.
** The test was conducted using calcium cyanamide.

DATA EVALUATION RECORD

Subject: "Determination of the Acute Oral ($AOLD_{50}$) Toxicity of Cyanamid in Albino Rats"

Test Material: Cyanamid L 500, described as a clear liquid. (This material is equivalent to Dormex or SKW-Cyanamid L 500.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: 2949 WN Anal. 2870 (1972)/February 7, 1973

Author: C. Engel

Classification: Minimum

Toxicity Category: Category II

Materials and Methods:

Twenty male and twenty female Wistar-derived albino rats obtained from the "Institutes" colony were distributed into four groups of five animals/sex/group. Males weighed 199 to 265 g and females weighed 106 to 165 g. The animals were fasted for 16 hours prior to treatment. The test material as a 5 percent (v/v) dilution in water was administered to the animals at 4, 5, 6, and 7 mL/kg (equivalent to 0.20, 0.25, 0.30 and 0.35 mL/kg of the undiluted test material). Rats were given stock diet and tap water ad libitum after administration of the test material. The animals were observed for 14 days for signs of toxicity. Necropsies were performed on all surviving animals. The LD_{50} was calculated by the method of Litchfield and Wilcoxon.

Results:

The following table provides information on the dose levels at which mortality occurred:

	Number Dead/Number Treated		
Dose 5% Dilution	Males	Females	Combined
4 mL/kg	1/5	0/5	1/10
5 mL/kg	4/5	0/5	4/10
6 mL/kg	5/5	2/5	7/10
7 mL/kg	5/5	1/5	6/10

Death usually occurred during the first day, however, a few rats died in the following 2 days. Death was preceded by convulsions. All surviving animals appeared to be healthy at the end of the observation period. No abnormal findings were observed at necropsy.

Conclusions: LD_{50} = 0.285* (0.250 to 0.325) mL/kg or approximately 300* mg/kg
*based on 100% Cyanamid L 500.

Toxicity Category: Category II.

Classification: Minimum.

Justification of Classification:

The data would not support a Guideline classification because the number of animals displaying toxic signs together with the duration and time of onset of the toxic signs were not provided in sufficient detail.

DATA EVALUATION RECORD

Subject: "Acute Dermal Toxicity Study ($ADLD_{50}$) with SKW Cyanamid L 500 in Rabbits"

Test Material: SKW Cyanamid L 500, a 50% aqueous solution. (This material has been identified as Dormex.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 4108/June 1973

Author: L. van Beek and H.C. Dreef-van der Meulen

Classification: Supplementary

Toxicity Category: Category III

Materials and Methods:

Eight male and eight female adult New Zealand White albino rabbits were divided into four groups of two animals/sex/group according to body weight. Initially, males weighed 2.37 to 2.93 kg and females weighed 2.56 to 2.90 kg. The hair from part of the trunk of the animals was clipped the day prior to the start of the experiment. The shaved area constituted 10 percent of the total body surface. The test material was applied at dose levels of 0.0 (control), 2.0, 4.0, or 6.0 mL/kg. Half of the animals received the test material on intact skin, the other animals received the test material on abraded skin. The treated area was covered with a thin layer of cellulose sheet and wrapped in polyethylene. After 24 hours of exposure the test material was removed from the skin with water and the skin was wiped with a towel. The animals were then caged individually in a room with a temperature of approximately 18 °C. Standard laboratory diet and tap water were available ad libitum. The animals were observed for a 2-week period for signs of toxicity, mortality, skin reactions, growth, and food and water intake. At the end of the observation period the following hematologic parameters were measured: hematocrit, hemoglobin concentration, erythrocyte count, and leucocyte count. Necropsies were conducted at the end of the study and the liver, kidneys, spleen, treated and untreated skin, and macroscopically abnormal stomachs were collected, fixed in 4% neutral buffered formaldehyde solution and examined.

Results:

The following table provides information on the dose levels at which mortality occurred:

	Number Dead/Number Treated		
Dose (ml/kg)	Males	Females	Combined
0.0	0/2	0/2	0/4
2.0	0/2	0/2	0/4
4.0	1/2	0/2	1/4
6.0	2/2	2/2	4/4

During or at the end of the exposure period the animals in the 2.0 and 4.0 mL/kg group exhibited apathy, dilation of the pupils, and erythema and edema of the treated skin. Hemorrhages at the site of treatment were more common on abraded skin than on intact skin. One male in the 4.0 mL/kg group had very severe paresis and was sacrificed when moribund. At the 6.0 mL/kg dose level, the animals had signs of apathy and dilation of the pupils. Slight erythema and very severe edema were observed. The rabbits were severely paretic and hemorrhages of the skin were apparent. Three of the 4 rabbits in the 6.0 mL/kg group died during the 24-hour exposure period. The fourth rabbit was sacrificed in a moribund condition at the end of the exposure period. After 1 and 2 weeks, the animals in the 2.0 and 4.0 mL/kg groups displayed scaliness of the treated skin and necrotic areas were present. There were no differences among control and treated animals with respect to body weight gain, food, and water consumption and hematology. At necropsy, the skin revealed treatment-related changes. Other treatment-related gross lesions included swollen livers with a distinct lobular pattern and hemorrhagic erosions of the stomach in animals that died or were sacrificed *in extremis*. Microscopic examination revealed acanthosis, parakeratosis, hyperkeratosis and edema of the skin, enlarged periportal hepatocytes in the liver, atrophy of the white pulp of the spleen, and hemorrhagic erosions of the stomach. The lesions of the liver, spleen, and stomach were observed only in animals that died or were sacrificed *in extremis*.

Conclusions: The dermal LD_{50} lies between 4.0 and 6.0 mL/kg.

Toxicity Category: Category III.

Classification: Supplementary.

Justification of Classification:

The study does not meet Minimum Data standards because too few animals/sex/dose were employed.

317
6

DATA EVALUATION RECORD

Subject: "Acute Inhalation Toxicity Study ($AILC_{50}$) with SKW Cyanamid L 500 in Rats"

Test Material: SKW Cyanamid L 500, a yellowish liquid stated to be a 50% aqueous solution of pure cyanamide. (This material has been identified as Dormex.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 4083/ May 1973

Author: A. Kruysse

Classification: Minimum

Toxicity Category: Category III

Materials and Methods:

Five male and five female, nine-week-old Wistar-derived albino rats were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, at Zeist, The Netherlands. Males weighed approximately 200 g and females weighed approximately 180 g. The animals were exposed to an atmosphere containing 2.0 g/m^3 of SKW Cyanamid L 500 for 4 hours. The atmosphere was generated using a CIVO-TNO-designed nebulizer. The nebulizer consisted of a Lechler Dr 0011 stainless steel nozzle atomizer, mounted on the stainless steel top of a 3/4 L Weck jar which contained the test material. The nebulizer produces a fine mist at a rate of 50 liters/min. The rats were individually housed in wire screen cages which were placed inside a 1.5 m^3 exposure chamber. Air samples were obtained through sampling ports by means of a fourfold fritted-glass absorber with approximately 80 mL of 0.1 N hydrochloric acid as the absorber. The cyanamide content was determined colorimetrically. Droplet size was determined by use of a cascade impactor. After the exposure, the rats were removed from the exposure chamber and observed for a period of 14 days. At the end of the 14-day observation period the animals were necropsied.

Results:

The animals showed a "lumback" behavior during the exposure and kept their eyes partly closed. The rats had rapid and shallow respiration with frequent coughing and swallowing. No deaths

occurred. The rats appeared to have completely recovered within a few hours after the exposure. No sign of injury was observed at necropsy. The concentration was stated to be 2.0 g/m^3 and 99 percent of the droplets were determined to be 1.5 um in diameter or less. The maximum particle size was 3 um.

Conclusions: $LC_{t50} > 2.0\ g/m^3$ (2.0 mg/liter) t = 4 hours.

Toxicity Category: Category III.

Classification: Minimum.

Justification of Classification:

Although fewer than three dose levels were employed, it was stated that the material was tested at the highest concentration possible. The material was tested for 4 hours. In addition, the analytical concentration and particle size were determined. The study can be interpreted as a "Limit Test" which was adequately conducted and is considered to be "Minimum."

DATA EVALUATION RECORD

Subject: "Primary Dermal Irritation/Corrosion Test with SKW-Cyanamid L 500 in Albino Rabbits"

Test Material: SKW-Cyanamid L 500, described as a colorless liquid. (On a cover sheet to this report, this material was identified as Dormex.)

EPA File Symbol: N/A - This submission was received in correspondence from S.R. Siemer to R.F. Mountfort dated November 12, 1985

Accession No.: N/A

Testing Facility: CIVO Institutes TNO
Zeist, The Netherlands

Report No./Date: B82-0061-4/1982

Author: L. van Beek

Classification: Minimum

Toxicity Category: Category I

Materials and Methods:

Six healthy adult New Zealand White albino rabbits had the hair removed from their backs with electric clippers. After 24 hours, 0.5 mL of the test material was placed on intact and abraded skin under a surgical patch measuring 1 inch x 1 inch. Adhesive tape was used to attach the patches to the application sites and the entire trunk of the rabbit was wrapped with an impervious material. After an exposure period of 4 hours the patches and test material were removed and the skin reactions evaluated. Forty-eight hours later the skin reactions were evaluated. The Draize method was used to grade the test sites. The animals were observed for 7 days.

Results:

After 4 hours of exposure the animals displayed "very slight to moderate erythema, slight ischemia, and slight to severe edema." After 52 hours the animals displayed "well-defined erythema, slight ischemia, distinct encrustation and very slight or slight edema." The presence of hemorrhages was suggested by the slightly purple color of the treated skin areas. After 1 week the major part of the treated skin showed slight to distinct necrosis. The average scores were 5.6 and 4.9 at 4 and 52 hours, respectively.

Conclusions: The test substance is corrosive.

320
9

Toxicity Category: Category I.

Classification: Minimum.

Justification of Classification:

Although readings of the skin were not taken at 24 and 72 hours, the results of the study clearly indicate that it is a corrosive substance. Therefore, the study is considered to be adequate and warrants a "Minimum" classification.

DATA EVALUATION RECORD

Subject: "Primary Skin Irritation Tests with Three Aqueous Dilutions of Alzodef in Albino Rabbits"

Test Material: Alzodef, described as a clear, colorless liquid stated to be a 50% aqueous dilution of cyanamide.

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: CIVO Institutes TNO
Zeist, The Netherlands

Report No./Date: V 84.090/230061 - February 1984

Author: L. van Beek

Classification: Invalid

Toxicity Category: Category IV

Materials and Methods:

In the protocol section of the report it is stated that twelve healthy adult New Zealand White albino rabbits were used to test one to four test substances; however, only six rabbits were used. Twenty-four hours prior to application of the test material the hair was removed from the backs of the animals with electric clippers. Six rabbits were treated on intact skin and six additional rabbits were treated on abraded skin. It is not clear whether the six rabbits that were actually used were treated on intact or abraded skin. One-half mL of the test substance was placed on the skin under a surgical patch measuring 1 inch x 1 inch. The test materials used were aqueous dilutions of Alzodef containing 1, 5, and 25 percent of cyanamide. The patches were attached to the application site with adhesive tape with an impervious material. After an exposure period of 24 hours the patches and the test material were removed and the skin reactions evaluated using the Draize method. In another section of the report the exposure period was stated to be 4 hours. A second reading was made 48 hours later using the CIVO-grading system. In another section of the report it was stated that skin reactions were evaluated at the end of the 4-hour exposure period, and then 24, 48, and 74 hours later, and after 1 week. The CIVO-grading system is described below:

Evaluation of Skin Reactions (CIVO)	Value
No reaction at all	0
Very slight scaliness	1
Distinct scaliness or very slight encrustation	2
Distinct encrustation	3
Distinct encrustation	4

Results:

The Alzodef dilutions containing 1 percent and 5 percent of cyanamide produced very slight erythema with or without very slight edema and some slight scaliness of the treated skin. The degree of irritation was greatest 4 hours after application of the test material. At 76 hours postapplication, only very slight irritation was present. No irritation was present after 7 days. The Alzodef dilution containing 25 percent cyanamide produced very slight erythema to severe erythema and very slight edema at 4 hours. Some slight scaliness was present. These effects cleared during the course of the 7-day period. No irritation was present on day 7.

Conclusions: The Alzodef dilutions containing 1, 5, and 25 percent cyanamide produce slight to moderate irritation. However, the conditions of the test could not be determined due to discrepancies in reporting.

Toxicity Category: Category IV.

Classification: Invalid.

Justification of Classification:

The study has errors in reporting the test conditions. The study may be upgraded provided the actual test conditions can be fully described. The petitioner should supply this information.

323
12

DATA EVALUATION RECORD

Subject: "Eye Irritation Test with SKW Cyanamid L 500 in Albino Rabbits"

Test Material: SKW Cyanamid L 500, described as a 50% aqueous solution of cyanamid. (This material has been identified as Dormex.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 4398/June 1974

Author: L. van Beek

Classification: Minimum

Toxicity Category: II

Materials and Methods:

The eyes of six New Zealand White albino rabbits were examined for defects prior to instillation of 100 mg of the test material into the everted lower lid of one eye of each rabbit. The upper and lower eyelids were then closed and held together for at least 1 second. The remaining eye served as a control. The eyes remained unwashed and were examined at 24, 48, 72 hours, and 7 days after instillation of the test material. Ocular reactions were read using a binocular magnifying glass and, if necessary, by staining the eyes of the animals with fluorescein. Reactions were scored using the Draize method.

Results:

The test material produced slight corneal opacity, mild iritis, moderate redness and moderate to severe swelling of the conjunctivae in all six rabbits after 24 hours. Some recovery occurred during the week; however, all animals had slight conjunctivitis on day 7.

Conclusions: The test material is an eye irritant.

Toxicity Category: II.

Classification: Minimum.

Justification of Classification:

The period of observation was only 7 days instead of 21 days even though minimal irritation was present at 7 days.

DATA EVALUATION RECORD

Subject: "Sensitization Test with SKW-Cyanamid F 1000 in Guinea Pigs (Maximization Test)"

Test Material: SKW-Cyanamid F 1000, described as a white crystalline material [purity?]

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: CIVO Institutes TNO
Zeist, The Netherlands

Report No./Date: V 82.096/220063 - March 1982

Authors: H.P. Til and D.C. Veldhuysen

Classification: Guideline (tentative).

Materials and Methods:

Fifteen SPF male albino guinea pigs weighing 282 to 325 g were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands, and allowed to acclimate to the laboratory for 2 days. The guinea pigs were housed in suspended stainless steel cages, fitted with wire mesh floors and fronts. The temperature was kept at 21 ± 1 °C, the relative humidity of 40 percent or greater and a 12-hour light/dark cycle was maintained. The guinea pigs were provided pelleted stock diet from Hope Farms (Woerden, The Netherlands) and tap water ad libitum. The study design used was the Guinea Pig Maximization Test. A preliminary study was conducted in order to determine the concentrations that were suitable for intradermal induction, for topical induction, and for topical challenge in the main study. A large area on the flanks of 3 to 6 guinea pigs were clipped free of hair. Subsequently, 0.1 mL portions of different dilutions of the test material in water were injected intradermally. Skin readings were made for the next few days. The dilution that caused slight to moderate irritation was used to make the intradermal injections. This material was 10 percent v/v of the test material in water. A second set of guinea pigs had their flanks clipped free of hair. Several 1 x 1 cm patches of filter paper containing different dilutions of the test material in vaseline were placed on the shaven skin and covered with a hypoallergenic paper bandage which was secured around the torso of the animal with elastic adhesive bandage. The dressing was left in place for 24 hours, then the animals were examined for skin irritation. The dilution that causes minimal irritation was used for topical induction (5% w/w in vaseline) and the dilution that was practically a non-irritant was used for topical challenge (2.5 and 1% w/w in vaseline).

In the main test, 15 animals were divided into 2 groups. The control group contained 5 animals and the test group contained 10 animals. An area of approximately 24 cm^3 of dorsal skin in the shoulder region was clipped free of hair. Three pairs of intradermal injections were made from front to back along the midline of the back. For the test animals, one pair of intradermal injections consisted of 0.1 mL of Freund's Complete Adjuvant (FCA). The second pair of injections consisted of 0.1 mL of 10 percent (v/v) of the test material in water. The third set of injections was 0.1 mL of a 1:1 mixture of 10 percent (v/v) of the test material in a mixture of FCA in water. The total volume of each preparation injected was divided between the left and right injection sites. For the control animals, one pair of injections consisted of 0.1 mL of FCA, the second pair of injections consisted of 0.1 mL of water, and the third pair of injections consisted of 0.1 mL of a 1:1 mixture of FCA and water. Skin reactions were scored 24 hours after treatment. One week after the intradermal injections were made the same area of the skin was shaved. For test animals, a 2 x 4 cm patch of Whatman No. 3 MM filter paper containing 5 percent (w/w) of the test material in vaseline was placed over the sites of the intradermal injections and covered with a piece of polyvinylchloride foil and a piece of "Leukopor" hypoallergenic paper bandage. Over the paper bandage was placed a 7.5 cm wide "Tensoplast" bandage. The dressing was left in place for 48 hours. The control animals were treated similarly with carrier only. Skin reactions were read after removal of the patches. Two weeks after topical induction an area of 5 x 5 cm on the left and right flank of each animal was clipped free of hair and shaved closely. For test animals, a "Silverpatch" was loaded with a 2.5 percent dilution of the test material in vaseline and placed on the right flank and a "Silverpatch" loaded with a 1 percent dilution of the test material in vaseline was placed on the left flank. The patches were covered with "Leukopor" bandages and held in place with "Tensoplast." Control animals received the same treatment. The patches were held in place for 24 hours. Skin readings were made immediately after removal of the patches and 24 and 48 hours later.

Results:

All animals appeared to be healthy throughout the test period. During the induction phase the following reactions were observed: with FCA - slight erythema and slight edema in all test and control animals; with 10 percent dilution of the test material in water - slight erythema and abscesses in all test animals; with water - no reactions in the control animals; with 10 percent dilution of the test substance in a mixture of FCA and water (1:1) - slight erythema, slight edema and abscesses on all test animals; with a mixture of FCA and water - slight erythema and slight edema in all control animals. During the induction phase the topical applications induced slight erythema in 4 of 10 test animals. Vaseline, when applied topically, did not induce skin

reactions. The challenge treatment with the 2.5 percent dilution of the test substance produced a well-defined to severe erythema in all 10 animals immediately after removal of the dressing. One of the controls reacted slightly at this time. After 24 and 48 hours the test animals still had positive reactions. The challenge treatment with the 1 percent dilution produced slight to moderate erythema in all test animals immediately after removal of the dressing. One control reacted slightly at this time. After 24 and 48 hours, 8 and 7 test animals, respectively, still showed slight to moderate erythema.

Conclusions: The test material is a strong skin sensitizer.

Classification: Guideline (tentative).*

*The sponsor should provide information on the purity and chemical composition of the test material.

DATA EVALUATION RECORD

Subject: "Range-Finding (28-Day) Toxicity Study with Cyanamid L 500 in Albino Rats"

Test Material: Cyanamid L 500 (Alzogur), described as a slightly turbid, bluish liquid. (This material is equivalent to Dormex.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 4387/ May 1974

Authors: H.P. Til, M.Th. Spanjers and C.A. van der Heijden

Classification: Supplementary

Materials and Methods:

Fifty male and fifty female weanling Wistar-derived rats from the CIVO-colony were divided according to body weight into 5 groups of 10 males and 10 females each. Mean male weight was 60.5 g and mean female weight was 58.5 g. Animals received 0, 100, 300, 1000 and 3000 ppm of Cyanamid L 500 in a stock diet for 28 days. Mean body weights and mean food consumption were reported weekly. Water intake was measured only during the first week. Hemoglobin concentration was measured in all rats at the end of week 4. After 28 days, all animals were sacrificed and necropsied. Liver and kidneys were weighed. Sections of the liver and kidney were obtained for histological examination.

Results:

One female in the 3000 ppm group died during the fourth week. Rats in the 3000 ppm group appeared to be in poor health during the first week. These animals were emaciated, had rough coats and decreased activity. There was a decrease in body weight gain in males and females in the 300, 1000, and 3000 ppm groups. Animals in the 3000 ppm group lost weight. Food consumption was decreased in males in the 300, 1000, and 3000 ppm groups and in females in the 1000 and 3000 ppm groups. Food efficiency was decreased in males and females in the 1000 and 3000 ppm groups. Water consumption was decreased in males and females in the 3000 ppm group. At 4 weeks, the hemoglobin concentration was decreased in both sexes in the 1000 and 3000 ppm groups. The relative weight of the liver was increased in males and females in the 3000 ppm groups. The relative weight of the kidneys was increased

in males and females in the 3000 ppm group. Males and females in the 3000 ppm group exhibited grossly pale livers at necropsy. On histological examination of the liver, all animals except the controls had evidence of hepatocellular injury. Extensive hydropic liver cell degeneration and ballooning of liver cells together with very marked bile duct proliferation was present in males and females in the 3000 ppm group and to a lesser extent in animals in the 1000 ppm group. Animals in the 300 and 100 ppm groups had slight to moderate enlargement of periportal hepatocytes, a mild degree of scattered liver cell necrosis and occasionally hydropic degeneration of isolated liver cells.

Conclusions: NOEL < 100 ppm.
LEL = 100 ppm (on the basis of hepatocellular degeneration).

Classification: Supplementary.

Justification of Classification:

The data were summarized. Individual animal data should have been submitted.

330
19

DATA EVALUATION RECORD

Subject: "Subchronic (90-Day) Toxicity Study with Cyanamid L 500 in Albino Rats"

Test Material: Cyanamid L 500 (Alzogur), described as a turbid, bluish liquid. (This material is equivalent to Dormex or SKW-Cyanamid L 500.)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 4595/January 1975

Authors: H.P. Til, R.B. Beems, and C.A. van der Heijden

Classification: Supplementary

Materials and Methods:

Forty male and forty female Wister-derived weanling albino rats obtained from the CIVO-colony were divided into groups of ten males and ten females each. Mean male and female body weights were 54.8 and 51.6 g, respectively, at initiation of the study. The animals received 0, 20, 60, or 180 ppm of Cyanamid L 500 in a stock diet. All diets were prepared once every 2 weeks and stored at room temperature. The rats were housed 5 per cage in screen-bottomed cages. The temperature was kept at 24 to 26 °C. Diets and tap water were available ad libitum. Rats were observed "regularly" for general appearance, condition, and behavior. Individual body weights were recorded weekly. Group food intake was measured during the first 4 weeks and from week 10 to 12. At week 12, blood was collected from 10 rats/sex/group. The following hematological parameters were measured: hemoglobin concentration, packed cell volume, erythrocyte count, and total and differential leukocyte counts. Urine samples were collected from 10 rats/sex/group in week 13. Urinalysis consisted of the appearance, pH, glucose, protein, occult blood, ketones, and a microscopic examination of the sediment of pooled samples. During week 14, blood samples were collected from all rats by decapitation. The following serum enzyme activities were measured: glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and alkaline phosphatase. Serum protein and serum albumin determinations were also made. At week 14 all animals were necropsied. The following organs were weighed: heart, kidneys, liver, spleen, brain, gonads, thymus, thyroid, and adrenals. The following tissues from animals in the control and high dose groups were fixed in 4 percent neutralized formaldehyde solution. The following hematoxylin and eosin stained paraffin sections were prepared for

microscopic examination: heart, kidneys, liver, spleen, brain, gonads, thymus, thyroid, adrenals, lung, trachea, salivary glands, prostate, epididymis, uterus, urinary bladder, skeletal muscle, thoracic aorta, esophagus, gastrointestinal tract (6 levels), pancreas, and axillary and mesenteric lymph nodes. Microscopic examination of tissues from rats in the low- and mid-dose group was restricted to liver and thyroids.

Results:

No deaths occurred and animals did not display any abnormal behavior. There were no differences in body weight gain, food consumption, or food efficiency among treated and control groups. There were no differences in hematological parameters, serum enzyme activities, serum protein or serum albumin values among treated and control groups. There were no differences in the urinalysis parameters among treated and control groups. The relative weight of the liver was slightly increased in males in the 180 ppm group and the relative weight of the thymus was decreased in females in the 180 ppm group. At necropsy, there were no pathological findings that could be attributed to administration of the test material. On microscopic examination of the tissues, the thyroids of 3 males and 2 females in the 180 ppm group and 1 male in the 60 ppm group had treatment-related changes consisting of morphological activation of the thyroid, seen as an increased number of small follicles lined by cuboidal epithelium and disappearance of colloid. In addition, the number of interfollicular cells was increased which was occasionally accompanied by a marked proliferation of follicular epithelial cells. Males in the 180 ppm group exhibited an increase in the number of small foci of necrotic liver cells with a slight degree of single liver cell necrosis.

Conclusions: NOEL = 20 ppm.
LEL = 60 ppm (based on histological changes in the thyroids of males).

Classification: Supplementary.

Justification of Classification:

The data were summarized. Individual animal data should have been submitted.

DATA EVALUATION RECORD

Subject: "Oral Embryotoxicity/Teratogenicity Study with an Agueous Cyanamide Solution (Content 49%) in New Zealand White Rabbits"

Test Material: Cyanamide, purity 49%, described as a light yellow liquid
Sample Code No. 250185

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073727

Testing Facility: Civo Institutes TNO
Zeist, The Netherlands

Report No./Date: V 84.444/240171/November 1984

Authors: H.B.W.M. Koeter and M.W. van Marwijk

Classification: Guideline

Materials and Methods:

Six-month-old virgin female and fertile male New Zealand White rabbits weighing 3060 to 4460 g were obtained from ENKI-Konijnenfarm, Someren, the Netherlands. Upon arrival the rabbits were examined for overt signs of illness and anomalies. The rabbits were housed individually in suspended steel cages with wire-mesh floors and fronts. Light was provided for a period of 12 hours/day; the temperature was kept at 18 $\pm$ 1 °C and the relative humidity was maintained at 40 to 70 percent. After an acclimatization period of 1 week, rabbits were artificially inseminated on weekdays with spermatozoa from male rabbits of proven fertility. Ovulation was induced by intravenous injection of luteinizing hormone. The day of insemination was considered to be day 0 of gestation. Inseminated females were assigned to each of four groups on rotation. The groups consisted of 24 to 25 females each which were administered 0, 4, 12, and 36 mg/kg of the test material during days 6 to 19 of gestation at a dosing volume of 2 mL/kg. The carrier was distilled water. The dosing solutions were prepared weekly and stored at 4 °C. Prior to administration the dosing solutions were stirred. Daily doses were changed to reflect daily body weight changes. The rabbits were observed daily for appearance and behavior. The rabbits were weighed on days 0, 6, 19, and 29 of gestation. Food consumption was determined in the following intervals: days 0 to 6, 6 to 19, and 19 to 29 of gestation. On day 29 of gestation, the rabbits were sacrificed by intravenous injection of 1 mL/kg Euthesate®. Both ovaries and uterus were exteriorized. The number of corpora lutea was recorded for each ovary and

both ovaries and the gravid uterus were weighed. The fetuses were removed from the uterus, weighed and examined for external malformations. Measurements were made on the length of the fetuses. The placentas of the live fetuses were weighed and examined for gross abnormalities. Early and late resorptions and dead fetuses were counted. The number of implantation sites in both uterine horns was recorded and the empty uterus was weighed. Half of the fetuses of each litter were decaptitated and the heads were fixed in Bouin's fixative and then free-hand sectioned using a modification of the Wilson technique. The bodies were examined by dissection for visceral abnormalities. Afterwards, the bodies were cleared and stained with Alizarin Red S for skeletal examination. The remainder of the pups were examined for visceral abnormalities by dissection and then the intact fetuses were cleared for skeletal examination. The preimplantation loss, postimplantation loss, and degree of ossification of fetal skeletions were calculated. The Student t-test was used to analyze the difference in the degree of ossification between the test and the control groups after using the arcsine transformation on the data. Statistical analysis of differences in body weight, food consumption, organ weights, litter data, fetal weights, and placenta weights were performed by using analysis of covariance, followed by Dunnett's multiple comparison test. Skeletal and visceral anomalies were evaluated by the Chi-square test.

Results:

During the study there were no effects on the appearance or behavior of the female rabbits. Body weight gain was decreased in rabbits administered 12 and 36 mg/kg during days 0 to 29 of gestation when compared to controls. One rabbit died in each of the control and 36 mg/kg groups. Preterm delivery occurred in one rabbit in the control group and in two rabbits in the 12 mg/kg group. The fertility indices ranged from 83.3 to 95.8 percent with no apparent compound-related effects. The gestation index ranged from 90 to 100 percent. There was an increase in the numbers of early resorptions and dead fetuses in the 36 mg/kg group. (Therefore, the postimplantation index was increased in the 36 mg/kg group. There was a decrease in the number of corpora lutea in animals in the treatment groups when compared to controls. However, historical control data submitted by the sponsor indicate that the number of corpora lutea in animals in the control group was high and that the number of corpora lutea in animals in the treatment groups was within normal limits. The weight of the gravid uterus and empty uterus was decreased in all treatment groups in a dose-response relationship. Mean fetal weights and fetal length were slightly decreased in the 36 mg/kg group. There was an increased incidence of small fetuses in the 36 mg/kg group when examined macroscopically. The incidence of small meningeal hemorrhages and/or hemorrhages

of the olfactory bulb, as well as the incidence of hemorrhagic gallbladder appendixes and disintegration of liver structure was increased in the 36 mg/kg group. The incidence of "minor eye anomalies," i.e., one or a few folds in the retinae, was increased in the 12 and 36 mg/kg groups. However, the increased incidence of increased folds in the retinae are considered to be an artifact (personal communication with Q. Bui, December 5, 1985).

Conclusions: Negative for teratogenicity
NOEL (maternal toxicity) = 4 mg/kg
LEL (maternal toxicity - decrease in body weight gain during gestation) = 12 mg/kg
NOEL (developmental toxicity) = 12 mg/kg
LEL (developmental toxicity - increase in early resportions and dead fetuses, decrease in fetal weight and size, increase in small meningeal hemorrhages and/or hemorrhages of the olfactory bulb and disintegration of liver structure). = 36/mg

Classification: Guideline.

Reviewed by: William B. Greear, M.P.H.
Section VII, Tox Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski, Ph.D.
Section VII, Tox Branch (TS-769C)

DATA EVALUATION RECORD

Study Type: Oncogenicity - Mouse

Tox. Chem. No.: 140

Accesion No.: 073727

MRID No.: Not available

Test Material: Calcium Cyanamide

Synonyms: Cyanamide, Cyanamid, Lime-Nitrogen, Calcium Carbimide, Aero Cyanamid Granular, Aero Cyanamid Special Grade, Calcium Cyanamid, Calcium Cyanamide, Cyanamid Granular, Cyanamid Special Grade

Study No.: (NIH) 79-1719

Sponsor: Siemer & Associates, Inc. for SKW Trostberg AG

Testing Facility: NCI Frederick Cancer Research Center
Fredrick, MD

Title of Report: Bioassay of Calcium Cyanamide for Possible Carcinogenicity

Author: Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

Report Issued: 1979

Conclusions: Classification: Supplementary.

Summary: Negative for oncogenicity.

A. Materials:

1. Test compound - Calcium cyanamide, described as a fine grey-black powder.

   Purity: 48 to 66%; contaminants 12 to 16% calcium oxide, 11 to 13% free carbon, 0 to 4% water, traces of selenium, nickel, and chromium.

2. Test animals - Species: mouse; Strain: $B_6C_3F_1$; Age: 4 weeks; Weight: males 18 to 22 g, females 17 to 21 g; Source: NCI Frederick Cancer Research Center (Frederick, MD).

B. Study Design:

1. Animal Assignment - Animals were assigned to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 100 Weeks Male	Female
1. Control	0	20	20
2. Low (LDT)	500	50	50
3. High (HDT)	2000	50	50

2. Diet preparation - Diets were prepared every 1 to 1 1/2 weeks and stored at 7 °C. Samples of treated diets were not analyzed for stability and concentration.

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: product-limit procedure of Kaplan and Meier (1958), Cox's method (1972), Tarone's (1975) extension of Cox's method, one-tailed Fisher exact test (Cox, 1970), Bonferroni inequality (Miller, 1966), Cochran-Armitage test with continuity correction (Armitage, 1971) and life-table methods.

5. Quality assurance was not provided.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Clinical examination and palpation for masses were performed each month.

Results

In male mice, 20/20 (100%) of the controls, 45/50 (90%) of the low-dose group, and 38/50 (76%) of the high-dose group lived to the end of the bioassay. In female mice, 18/20 (90%) of the controls, 43/50 (86%) of the low-dose group, and 46/50 (92%) of the high-dose group lived to the end of the bioassay. Survival was decreased in the high-dose male group.

2. Body weight - Animals were weighed at least once per month.

Results - Males in the low- and high-dose groups and females in the high-dose group exhibited slight decreases in body weight gain when compared to controls. Body weights of animals were elevated in an unusual manner at 10 weeks.

3. Food consumption and compound intake - data were not obtained.

4. Ophthalmalogical examinations - were not performed.

5. Blood was not collected.

6. Urinalysis - was not performed.

7. Sacrifice and pathology - All animals that died and that were sacrificed on schedule were subjected to a gross pathological examination and the CHECKED (X) tissues were collected for histological examination.

X		X		X			
	Digestive System		Cardiovasc./Hemat.		Neurologic		
	Tongue		Aorta	X	Brain		
X	Salivary glands	X	Heart		Perip. nerve		
X	Esophagus	X	Bone marrow		Spinal cord (3 levels		
1	Stomach	X	Lymph nodes	X	Pituitary		
1	Duodenum	X	Spleen		Eyes (optic n.)		
1	Jejunum	X	Thymus	Glandular			
1	Ileum	Urogenital		X	Adrenals		
1	Cecum	X	Kidneys		Lacrimal gland		
1	Colon	X	Urinary bladder	X	Mammary gland		
	Rectum	X	Testes	X	Parathyroids		
X	Liver		Epididynmides	X	Thyroids		
	Gallbladder	X	Prostate	Other			
X	Pancreas		Seminal vesicles		Bone		
Respiratory		X	Ovaries		Skeletal Muscle		
X	Trachea	X	Uterus	X	Skin		
X	Lung			X	All gross lesions and masses		

1 - Examination stated to include "small and large" intestine.

Peripheral blood smears were also prepared.

Results

a. Organ weights - not provided.
b. Gross pathology - not provided.
c. Microscopic pathology

1. Non-neoplastic - unremarkable.
2. Neoplastic - Two tumor types appeared to be increased in the treated mice. There was an increased incidence of malignant lymphoma in treated female mice and an increased incidence of hemangiosarcoma in males in the high-dose group. The incidence of malignant lymphoma and leukemia is provided in the following table:

Incidence of Malignant Lymphoma and Leukemia

	Control		Low-Dose		High-Dose	
Tumor Type	M	F	M	F	M	F
Malignant Lymphoma, NOS	0/20(0%)	0/20(0%)	0/50(0%)	1/46(2%)	1/50(2%)	3/50(6%)[2]
Malignant Lymphoma, histiocytic	1/20(5%)	1/20(5%)	4/50(8%)	9/46(19%)	1/50(2%)	12/50(24%)[2]
Malignant Lymphoma, lymphocytic	0/20(0%)	0/20(0%)	0/50(0%)	0/46(0%)	1/50(2%)	1/50(2%)
Malignant Lymphoma, mixed	0/20(0%)	0/20(0%)	0/50(0%)	0/46(0%)	0/50(0%)	0/50(0%)
Lymphocytic Leukemia	0/20(0%)	0/20(0%)	0/50(0%)	1/46(2%)	0/50(0%)	0/50(0%)
Monocytic Leukemia	0/20(0%)	0/20(0%)	0/50(0%)	0/46(0%)	3/50(6%)	2/50(4%)
Total	1/20(5%)	1/20(5%)	4/50(8%)	11/46(24%)[1]	6/50(12%)	18/50(36%)

1- The summary table indicates 10/46 with malignant lymphoma; however, in the body of the report the incidence is stated to be 11/46. In the statistical analysis, the incidence of malignant lymphoma and leukemia was reported to be 11/46.

2 - It could not be determined from the summary table whether the incidence of malignant lymphoma (NOS) and malignant lymphoma (histiocytic) was 3/50 and 12/50, or 4/50 and 11/50, respectively.

The results of the Cochran-Armitage test indicate a dose-related trend ($p = 0.008$) in the incidence of lymphoma or leukemia in female mice. When the Fisher exact test was used to compare the difference between the incidence of lymphoma or leukemia in high-dose and control female mice a p value of 0.006 was obtained.

The incidence of hemangiosarcomas is provided in the following table:

Incidence of Hemangiosarcomas

	Control	Low-Dose	High-Dose
Male	1/20(5%)	2/50(4%)	10/50(20%)
Female	0/20(0%)	0/46(0%)	1/50(2%)

The results of the Cochran-Armitage test indicate a dose-related linear trend ($p = 0.006$) in the incidence of hemangiosarcomas of all sites in male mice. The results of the Fisher exact test are not significant.

D. Discussion/Summary:

Survival was decreased in the high-dose male group. Males and females in the high-dose group and males in the low-dose group exhibited a slight decrease in body weight gain when compared to controls. The results of the histopathology examination revealed an increase in the incidence of malignant lymphoma and leukemia in treated females. The incidence was 1/20(5%), 11/46(24%), and 18/50(36%) in the control, low- and high-dose groups, respectively. There was a dose-related trend ($p = 0.009$) and in a direct comparison the incidence of these tumors in the high-dose group was significantly higher ($p = 0.006$) than that in the control group. The incidence of lymphomas or leukemias in historical control female $B_6C_3F_1$ mice at the testing laboratory was 67/324(21%).

The incidence of lymphoma-leukemia in $B_6C_3F_1$ mice has been reported for five laboratories conducting studies for NCI (Tarone et al., 1981). The mean and range of the incidence of these tumors in female mice in studies performed by the laboratories are provided below:

Percentage of Tumors (Lymphomas-Leukemias) in Female $B_6C_3F_1$ Mice at Five Laboratories

	Laboratory No. 1	2	3	4	5
Mean	25.4	30.4	17.0	23.0	22.7
Range	8-42	22-41	12-25	5-45	10-42

It is apparent on examining the data that the incidence of lymphoma/leukemia in the female control group is very low compared to the incidence observed in historical control female $B_6C_3F_1$ mice at the laboratory that conducted the study, as well as at a number of other laboratories. This suggests that the incidence of these tumors in the female control group was abnormally low and may be responsible for the statistical significance of the results. Therefore, it is concluded that the increase in the incidence of lymphoma/leukemia in females in the high-dose group is probably without biological significance.

There was also an increase in the incidence of hemangiosarcoma in males in the high-dose group. The incidence was 1/20(5%), 2/50(4%), and 10/50(20%) in the control, low- and high-dose groups, respectively. There was a dose-related trend ($p = 0.006$); however in direct comparisons, incidences in the individual treated groups were not significantly higher than those in the control group. The incidence of hemangiosarcomas in historical control male $B_6C_3F_1$ mice at the testing laboratory was 13/323(4%). The highest incidence observed in any male control group at the testing laboratory was 2/19(10%). The incidence of hemangiosarcoma in 2,343 male $B_6C_3F_1$ mice has been reported to range from 0 to 10 percent with a mean of 2.6 percent in studies conducted for NCI (Goodman et al., 1985).

Tumors of the vascular system are relatively common in aging $B_6C_3F_1$ mice. Although the incidence of hemangiosarcoma was twice the incidence observed in historical control mice, there was no statistical difference between the incidence observed in the treated and control mice. Therefore, the increase in incidence of hemangiosarcoma in males in the high-dose group cannot be attributed to administration of the test material.

A 7-week subchronic feeding study was initially conducted in order to estimate the maximum tolerated dose (MTD). Groups of 5 males and 5 females were administered 0, 1500, 3000, 4000, 8000, 10,000, 16,000 and 30,000 ppm of calcium cyanamide in the diet. Body weight gain was decreased at all dose levels. No deaths occurred at doses up to and including 16,000 ppm. One hundred percent mortality was observed at 30,000 ppm. Trace amounts of bile-duct hyperplasia were observed in male and female mice in the 16,000 ppm dose group. Periportal hepatocytes with pale-staining vacuolated cytoplasm were seen in male mice. Focal hepatic necrosis was present in four female mice. Body weight was decreased 7 to 8 percent and 13 to 15 percent in males and females, respectively, in the 1500, 3000 and 4000 ppm groups. Ten percent depression in body weight was a major criterion for estimation of the MTD in mice using least squares regression of probit analysis. A MTD dose was used in the chronic study.

The study suffers from several flaws. Inadequate numbers of animals were used in the control groups. It is unknown whether the test material was stable in the diet because the test diets were not assayed for concentration or homogeneity. Time-to-tumor formation data were not provided. Individual pathology sheets were not provided, therefore, associations between tumor formation and deaths could not be established. Descriptions of the various neoplasms (e.g., degree of malignancy, etc.) were not provided. In addition, growth curves appear to be in error for both males and females at week 10.

Oncogenicity: Negative

Core Classification: Supplementary for the above stated reasons.

It should be noted that the mice were housed in an animal room in which seven other bioassays were in progress. The studies were conducted on 1) (2-chloroethyl) trimethylammonium chloride; 2) 2,4-diaminotoluene; 3) lead dimethyldithiocarbamate; 4) N-nitrosodiphenylamine; 5) phthalamide; 6) piperonyl sulfoxide; and 7) 2,4,5-trimethylaniline. This could have provided the opportunity for cross-contamination.

References

1. Goodman, D.G., Boorman, G.A. and Strandberg, J.D. (1985) Selection and use of the $B_6C_3F_1$ mouse and F344 rat in long-term bioassays for carcinogenicity. In: Handbook of Carcinogen Testing.

2. Tarone, R.E., Chu, K.C. and Ward, J.M. (1981) Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6N X C3H/HeN)F_1 ($B_6C_3F_1$) mice. J.NCI 66(6):1175-1181.

Reviewed by: William B. Greear, M.P.H.
Section VII, Tox Branch (TS-769C)
Secondary Reviewer: Albin B. Kocialski, Ph.D.
Section VII, Tox Branch (TS-769C)

DATA EVALUATION RECORD

Study Type: Oncogenicity - Rat

Tox Chem No.: 140

Accession No.: 073727

MRID No.: Not available

Test Material: Calcium Cyanamide

Synonyms: Cyanamide, Cyanamid, Lime-Nitrogen, Calcium Carbimide, Aero Cyanamid Granular, Aero Cyanamid Special Grade, Calcium Cyanamid, Calcium Cyanamide, Cyanamid Granular, Cyanamid Special Grade.

Study No.: (NIH) 79-1719

Sponsor: Siemer & Associates, Inc. for SKW Trostberg AG

Testing Facility: NCI Frederick Cancer Research Center
Frederick, MD

Title of Report: Bioassay of Calcium Cyanamide for Possible Carcinogenicity

Author: Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

Report Issued: 1979

Conclusions: Classification: Supplementary.

Summary: Negative for oncogenicity.

A. Materials:

1. Test compound - calcium cyanamide, described as a fine grey-black powder; purity: 48 to 66 percent; contaminants, 12 to 16 percent calcium oxide, 11 to 13 percent free carbon, 0 to 4 percent water, traces of selenium, nickel and chromium.

2. Test animals - Species: rat; Strain: F344; Age: 4 weeks; Weight: males 90 to 105 g, females 80 to 95 g; Source: NCI Frederick Cancer Research Center (Frederick, MD).

B. Study Design:

1. Animal assignment - Animals were assigned to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study 107 Weeks Male	Female
1. Control	0	20	20
2. Low (LDT)	100	50	50
3. High (HDT)	200	50	50

2. Diet preparation - Diets were prepared every 1 to 1 1/2 weeks and stored at 7 °C. Samples of treated diets were not analyzed for stability and concentration or homogeneity.

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: product-limit procedure of Kaplan and Meier (1958), Cox's method (1972), Tarone's (1975) extension of Cox's methods, one-tailed Fisher exact test (Cox, 1970), Bonferroni inequality (Miller, 1966), Cochran-Armitage test with continuity correction (Armitage, 1971) and life-table methods.

5. Quality assurance was not provided.

C. Methods and Results:

1. Observations - Animals were inspected twice daily for signs of toxicity and mortality. Clinical examination and palpation for masses were performed each month.

   Results - In male rats, 14/20(70%) of the controls, 35/50(70%) of the low-dose group, and 39/50(78%) of the high-dose group lived to the end of the bioassay. In female rats, 18/20(90%) of the controls, 41/50(82%) of the low-dose group, and 41/50(82%) of the high-dose group lived to the end of the bioassay. Survival was unaffected by treatment.

2. Body weight - Animals were weighed at least once per month, except for weeks 50 to 80.

   Results - Mean body weights of rats in the high-dose group were slightly lower than those of the controls.

3. Food consumption and compound intake - data were not obtained.

4. Ophthalmological examinations - were not performed.

5. Blood was not collected.

6. Urinalysis - was not performed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination.

X		X		X	
	Digestive System		Cardiovasc./Hemat.		Neurologic
	Tongue		Aorta	X	Brain
X	Salivary glands	X	Heart		Perip. nerve
X	Esophagus	X	Bone Marrow		Spinal cord (3 levels
X	Stomach	X	Lymph nodes	X	Pituitary
1	Duodenum	X	Spleen		Eyes (optic n.)
1	Jejunum	X	Thymus		Glandular
1	Ileum		Urogenital	X	Adrenals
1	Cecum	X	Kidneys		Lacrimal gland
1	Colon	X	Urinary bladder	X	Mammary gland
	Rectum	X	Testes	X	Parathyroids
X	Liver		Epididymides	X	Thyroids
	Gallbladder	X	Prostate		Other
X	Pancreas		Seminal vesicles		Bone
	Respiratory	X	Ovaries		Skeletal muscle
X	Trachea	X	Uterus	X	Skin
X	Lung			X	All gross lesions and masses

1 - Examination included "small and large" intestine. Peripheral blood smears were also prepared.

Results

a. Organ weights - not provided.
b. Gross pathology - not provided.
c. Microscopic pathology.

1. Non-neoplastic - There was an increased incidence of dilation of the ducts and hyperplasia of the mammary gland in males in the high-dose group. Hyperplasia of the mammary gland was also observed in the male low-dose group.

2. Neoplastic - There was a slight increase in the incidence of pheochromocytoma in male rats in the high-dose group. The incidence of pheochromocytoma (benign and malignant) was 4/20(20%), 10/49(20%), and 15/50(32%) in the control, low-, and high-dose groups, respectively. The results of the statistical analysis using the Cochran-Armitage and Fisher exact test were not statistically significant.

D. Discussion:

Survival was unaffected by compound administration. Mean body weights of male and female rats in the high-dose group were slightly lower than controls. There was an increased incidence of dilation of the ducts and hyperplasia of the mammary gland in males in the high-dose group. Hyperplasia of the mammary gland was also observed in the male low-dose group. The neoplasms observed and their distribution were similar in control and treated rats.

Two 7-week subchronic feeding studies were initially conducted in order to estimate the maximum tolerated dose (MTD). In one study, groups of 5 males and 5 females were administered 0, 1500, 3000, 4000, 8000, 10,000, 16,000 and 30,000 ppm of calcium cyanamide in the diet. In a second study, groups of 5 males and 5 females were administered 0, 400, 600, 800, 900, 1000, 1200 and 1500 ppm in the diet. Body weight gain was decreased at all levels and 100 percent mortality was observed at dose levels above 400 ppm. Trace to moderate amounts of bile-duct hyperplasia were observed in rats dosed at 1500, 3000, and 4000 ppm. A very slight to moderate increase in extramedullary hematopoiesis was observed in the spleen of males and females. Marked diffuse hyperplasia of the thyroid was observed at the 4000 ppm dose level. Thyroid hyperplasia was also observed in rats in the 400, 600, 800, 900, 1000, 1200, 1500 and 3000 ppm groups. A MTD dose was used in the chronic study.

The design of the study is flawed in that the number of animals in the matched control group (20 rats/sex) is insufficient and reduces the sensitivity of the test. Additionally, analysis of dietary mixtures was not performed. Body weights of rats were not provided for months 15 through 18. Individual pathology sheets were not provided, therefore, time-to-tumor data were not available and associations between tumor formation and deaths could not be made.

It should be noted that the rats were housed in an animal room in which two other bioassays were in progress. The studies were conducted on 1) 4-chloro-o-toluidine hydrochloride (CAS 3165-93-3), and 2) N-nitrosodiphenylamine (CAS 86-30-6). This could have provided the opportunity for cross-contamination.

Oncogenicity: Negative.

Core Classification: Supplementary for the above stated reasons.

DATA EVALUATION RECORD

Subject: "Evaluation of Two Products CCA and CA in the Salmonella/microsomal Mutagenicity Test"

Test Material: CCA [purity not stated] (described as a greyish granular material)
CA [purity not stated] (described as a colorless liquid)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073727

Testing Facility: Centraal Instituut Voor Voedingsonderzoek Zeist, The Netherlands

Report No./Date: R 5707/ June 1978

Author: M.I. Williams

Classification: Unacceptable

Materials and Methods:

Salmonella typhimurium (his$^-$) strains TA1535, TA1537, TA1538, TA98, and TA100 were obtained from Dr. Bruce Ames, Berkeley, California. The mutagens, N-nitrosomorpholine and benzo(a)pyrene, were used as positive controls to test the sensitivity of the strains to revert to histidine prototrophy in the absence and presence of metabolic activation (rat S-9). The strains were also checked for their histidine requirement, for sensitivity to crystal violet and deoxycholate, and for resistance to ampicillin. The metabolic activation system used was an S-9 mix made from Aroclor 1254 induced rat liver plus cofactors, as described by Ames (1975). The amount of S-9 mix used throughout the study in activated cultures was 50 ul/plate. The metabolic activation properties of the liver homogenate was checked in a preliminary assay with S. typhimurium strain TA98 and 4-aminobiphenyl.

One-tenth mL of a fully grown culture of the tester strain or 0.1 mL of an "appropriate" dilution/suspension of the test material and the liver microsome system was added to 2.5 mL molten soft agar. CCA was tested at levels of 4, 20, 100, 500, and 1000 ug/0.1 mL DMSO with and without activation. CA was tested at levels of 0.004, 0.02, 0.1, 0.5, and 1 uL/0.1 mL DMSO with and without metabolic activation. The ingredients were mixed and then poured onto minimal glucose agar plates. After the top agar had hardened, the plates were incubated at 37 °C for 3 days. The number of colonies were counted and the background lawn of bacteria was examined. All determinations were made in

triplicate. N-nitrosomorpholine was used in nonactivated cultures as a positive control at levels of 3 and 6 ug/0.1 mL DMSO for strains TA1535 and TA100. Benzopyrene was used as a positive control in activated cultures at levels of 1 and 2.5 ug/0.1 mL DMSO for strains TA1537, TA1538, and TA98.

Results:

It was reported that "incorporation of up to 1000 ug CCA per plate or of 0.004 up to 1 uL CA per plate did not increase the number of his^+ revertants in any of the 5 tester strains either in the presence or in the absence of metabolic activation." No toxicity was observed when CCA was tested at up to 1000 ug/plate and when CA was tested at up to 1 uL/plate.

Conclusions: The test material was not adequately tested. Only summary data were submitted. Individual plate scores were conspicuously absent.

Classification: Unacceptable.

Justification of Classification:

1. Individual plate scores were not presented for evaluation.

2. For CA, the test material should have been tested at up to 50 ul/plate, since no toxicity was observed at the highest dose tested of 1 uL/plate.

3. No toxicity was observed when CCA was tested at 1000 ug/plate, therefore, a higher dose level (5000 ug/plate) should have been tested.

Comment:

The petitioner should also completely identify the composition of the test materials (provide percentages of ingredients) especially purity of the active ingredients.

DATA EVALUATION RECORD

Subject: "Evaluation of 'Kalkstickstoff' and 'Thioharnstoff' in the Micronucleus Test"

Test Material: Kalkstickstoff (equivalent to calcium cyanamide technical, approximately 97% pure), described as a greyish black powder
Thioharnstoff (purity not stated), described as a white crystalline solid

Positive Control: Trenimon

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073727

Testing Facility: Centraal Instituut Voor Voedingsonderzoek, Zeist, The Netherlands

Report No./Date: R 6012/ February 1979

Author: M.I. Williams

Classification: Unacceptable

Materials and Methods:

Fifteen male and fifteen female Wistar rats were obtained from the Central Institute for the Breeding of Laboratory Animals TNO, Zeist, The Netherlands. After the rats were acclimated to the laboratory for an "appropriate" period of time, the rats were distributed according to body weight into 3 groups of 5 males and 5 females each. After 14 to 15 hours of fasting, the rats were administered one-fifth of the LD_{50} of the appropriate test material in 5 mL H_2O/kg bw by gavage. The LD_{50} values for Thioharnstoff and Kalkstickstoff were reported to be 1750 and 765 mg/kg, respectively. Administration of the dose was repeated after 24 hours. The amount of test material administered was: 2 x 5 mL of 3.06% Kalkstickstoff (total 700 mg) and 2 x 5 mL of 7% Thiokarnstoff (total 306 mg). The control group was treated similarly except that the rats were administered water. The positive control group was administered 0.0625 mg of Trenimon (2,3,5-tris-ethyleneiminobenzoquinone) twice by the intraperitoneal route of administration. Six hours after dosing, the animals were sacrificed by decapitation. Bone marrow from the femur was obtained and centrifuged with fetal calf serum. The excess serum was removed and the cells were resuspended by gently mixing. Five slides were prepared for each animal. The smears were air dried, fixed in methanol and stained in May-Gruenwald solution followed by Giemsa. Four hundred erythrocytes were examined on each slide and the number of micronucleated polychromatic erythrocytes and the ratio of poly- and normochromatic erythroyctes was recorded.

Results:

No mortality or toxic signs were observed in the animals during the exposure period. In addition, cytoxicity was not observed. The incidence of micronucleated erythrocytes was comparable among treatment and vehicle control groups. In the positive group there was an increase (approximately 7X) in the incidence of micronucleated eythrocytes and a decrease in the percentage of polychromatic erythrocytes.

Conclusions: The test material was not adequately tested to levels of clinical cytotoxicity.

Classification: Unacceptable.

Justification of Classification:

1. Only summary data were presented. Individual animal data should have been submitted for evaluation.

2. A higher dose level should have been tested (one-half the LD_{50} or greater), so that animal toxicity or cytotoxicity would be observed at the highest dose level tested.

3. Data were not submitted on the LD_{50} studies of the test material.

DATA EVALUATION RECORD

Subject: "An Investigation into the Sister Chromatid Exchange Induction in Chinese Hamster Ovary Cells by a Sample of 'Kalkstickstoff'"

Test Material: Kalkstickstoff 23% N, described as a black powder that was stated to be a mixture of calcium cyanamide and carbon (equivalent to calcium cyanamide technical, approximately 97% pure)

Positive Control: Trenimon, a direct mutagen, and cyclophosphamide (metabolic activation required)

EPA File Symbol: 54555-EUP-R/5G3283

Accession No.: 073726

Testing Facility: Central Institute for Food and Nutrition Research (CIVO-TNO)
Zeist, The Netherlands

Report No./Date: CL/78/120/February 2, 1979

Author: W.K. de Raat

Classification: Unacceptable

Materials and Methods:

Chinese hamster ovary cells were inoculated into 10 mL portions of a medium (Ham's Flo medium supplemented with 15% of newborn calf serum, 50 mg/L streptomycin, and 50 mg of sodium penicillin G) contained in 250 mL flasks and incubated for 4 hours. In tests with metabolic activation 0.2 mL of S-9 mix from Aroclor 1254 treated rats, 0.2 mL of the test material in DMSO, and 0.5 mL of a coenzyme solution in sodium phosphate buffer of pH 7.4 containing 8 mmol of $MgCl_2$, 23 mmol of KCl, 4 mmol of NADP and 5 mmol of glucose-6-phosphate per liter was used. In tests without activation, only the test material was added. The test material was prepared in three ways: 1) the test material in DMSO was hand shaken, 2) the test material in DMSO was shaken for 20 hours in a shaking machine and the undissolved particles were removed by centrifugation, and 3) the test material in DMSO was shaken for 135 minutes in a shaking machine. The dose levels of test material used were 0, 10, 50, 250, and 500 mg/L.

When the cells had been exposed for 1 hour, the medium was replaced with one containing 10 mmol of 5-bromodeoxyuridine per liter. The flasks were then wrapped in aluminum foil. Twenty-four hours later the cells were harvested by the addition of trypsin. (Three hours prior to harvesting, colchicine was added

at a level of 2 ug/10 mL to arrest mitosis at metaphase.) After harvesting, the cells were treated for 7 minutes with 0.075 M KCl, fixed with a 3:1 mixture of methanol and acetic acid and transferred to slides. The cells were stained with Hoechst 33258 fluorochrome, exposed to white light for 5 hours and then stained with Giemsa. One slide was prepared from the contents of each flask and the number of SCE in 20 metaphases on each slide was scored.

Results:

The test material failed to induce an increase in SCE per metaphase at dose levels ranging from 10 to 500 mg/L. The highest dose level (500 mg/L) represents 330 mg/L of calcium cyanamide. The test material did not dissolve completely in 0.5 or 1 percent DMSO.

Conclusions: The test material was not adequately tested to levels of cytotoxicity.

Classification: Unacceptable.

Justification of Classification:

1. A greater number of cells should have been scored per treatment. Only twenty cells were scored per slide. Twenty-five cells should have been scored per replicate (with 2 replicates) for a total of at least 50 cells per treatment.

2. Replicate slides should have been prepared and scored.

3. Only summary data were provided. Individual cell scores were not provided.

LITERATURE ARTICLES

Several literature articles were submitted for evaluation. These articles have not been evaluated because of the lack of sufficient data. However, the author's summaries are provided below as a source of information.

1. Menargues, A., Obach, R., and Valle's J.M. (1984) An evaluation of the mutagenic potential of cyanamide using the micronucleus test. Mutation Research 136:127-129.

   "The bone-marrow micronucleus assay was used in mice to evaluate the mutagenic potential of cyanamide administered per os at doses of 10, 49 and 247 mg/kg. Bone-marrow smears were examined to find the incidence of micronucleated cells in 1000 polychromatic erythrocytes (PCE) and 1000 normochromatic erythrocytes (NCE). The ratio of NCE/PCE was also scored. No increase in the incidence of micronucleated polychromatic erythrocytes and no difference in the ratio NCE/PCE for the groups treated with cyanamide (10 and 49 mg/kg) were observed. The group treated with the highest dose of cyanamide (247 mg/kg) did, however, show an NCE/PCE ratio lower than the control group ($p < 0.05$)."

2. Shirota, F.N., Nagasawa, H.T., Kwon, C.H., and Demaster, E.G. (1984) N-acetylcyanamide, the major urinary metabolite of cyanamide in rat, rabbit, dog and man. Drug Metabolism and Disposition 12(3):337-344.

   "The structure of the major urinary metabolite of cyanamide, the active component of the alcohol deterrent agents Temposil, Dipsan and Abstem, in rats, rabbits, and dogs has been established as N-acetylcyanamide by its identity with chemically synthesized N-acetylcyanamide, and by conversion of the metabolite and its synthetic product to identical derivatives, viz., to N-benzyl-N-acetyl-cyanamide and to N-(p-nitrobenzyl)-N-acetyl-cyanamide. The latter derivatives were analyzed by pulsed positive/negative ion chemical ionization mass spectroscopy. Urine from patients receiving cyanamide as a treatment mode was shown to contain N-acetylcyanamide by chemical ionization mass spectrometric analysis of the isolated p-nitrobenzyl derivative, thereby establishing that N-acetylcyanamide is also a metabolite in man. The major portion (87%) of the first 27-hour urinary radioactivity excreted by the dog after receiving a low dose of [^{14}C]-cyanamide (0.04 mmol/kg,po) was N-acetylcyanamide, as determined by inverse isotope dilution and measurement of the specific radioactivity of its N-p-nitrobenzyl derivative."

"This indicates that at low doses acetylation is also a major route of biotransformation of cyanamide in the dog. Hepatic N-acetyltransferase, isolated from the rabbit and dog, catalyzed the transfer of the acetyl group from acetyl-S-CoA to [^{14}C]-cyanamide producing N-acetyl [^{14}C]-cyanamide. The enzyme isolated from the liver of a rapid acetylator phenotype rabbit was twice as effective as the dog enzyme in catalyzing this transfer. Thus, the enzyme responsible for this biotransformation of cyanamide is an acetyl-S-CoA-dependent N-acetyl-transferase."