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TOXIC SUBSTANCES

DATE: March 26, 2001

MEMORANDUM

SUBJECT: ***Hydrogen Cyanamide*** - Report of the Hazard Identification Assessment Review Committee.

FROM: John E. Whalan, Toxicologist.
Registration Action Branch 2
Health Effects Division (7509C)

John E Whalan 3-26-01

THROUGH: Jess Rowland, Co-Chair
and
Elizabeth Doyle, Co-Chair
Hazard Identification Assessment Review Committee
Health Effects Division (7509C)

E A Doyle for
E A Doyle

TO: John E. Whalan, Risk Assessor
Registration Action Branch 2
Health Effects Division (7509C)

PC Code: 014002

On January 10, 2001 and January 18, 2001 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for hydrogen cyanamide with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to hydrogen cyanamide was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. This document is a summation of the HIARC's decisions at both meetings.

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Committee Members in Attendance

Members present were:

William Burnam
Elizabeth Doyle
Pamela Hurley
Elizabeth Mendez
David Nixon
Ayaad Assaad
Yung Yang
Jess Rowland
Brenda Tarplee
and Jonathan Chen

Member(s) in absentia: None

Data evaluation prepared by: John E. Whalan, RAB2

Also in attendance were: Steven Weiss and William Greear of HED

Data Evaluation / Report Presentation

John Whalan 3-26-01
John E. Whalan, Toxicologist

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1. INTRODUCTION

On January 10, 2001 the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for hydrogen cyanamide with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to hydrogen cyanamide was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The HIARC met again on January 18, 2001 to select a target MOE for inhalation exposures. This document is a summation of the HIARC's decisions at both meetings.

A meeting of HED scientists was held on August 23, 1993 to discuss the various classification options for the use of hydrogen cyanamide on dormant grape vines. This workgroup concluded that, "...based on the anticipated total metabolism of hydrogen cyanamide into the general carbon pool of the grape, and the common use of hydrogen cyanamide and its precursor, calcium cyanamide, as fertilizers, the proposed use of hydrogen cyanamide on dormant grape vines would be classified as a non-food use." (G. Jeffrey Herndon memorandum, August 26, 1993)

A section 3 petition is pending for hydrogen cyanamide use on grapes, apples, blueberries, cherries, peaches, nectarines, and plums. The HIARC selected short and intermediate-term dermal and inhalation occupational endpoints. Since hydrogen cyanamide is a non-food-use, no oral RfDs are needed.

Hydrogen cyanamide is a plant growth regulator which causes chemical vernalization and uniform bud break. As a crystalline solid, it is combustible and deliquescent. All agricultural formulations (Dormex®, Cyanamide, Carbimide, Carbodimide, Amidocyanogen, Cyanogenamide, SKW 83010, and Alzodef®), contain 51% hydrogen cyanamide and 49% inert ingredients, and this is the material tested in all toxicity studies. All products are applied as a coarse droplet spray to dormant grapes and various stone fruit which have not received an adequate number of winter chilling hours, or to promote earlier budbreak even with sufficient winter chilling.

Hydrogen cyanamide is produced by the hydrolysis of calcium cyanamide, the only other related chemical. In moist soil, hydrogen cyanamide readily hydrolyzes into urea, ammonium, nitrate, dicyanodiamide, guanylurea, and guanidine. Hydrogen cyanamide and calcium cyanamide have both been used as sources of nitrogen fertilizer dating back to the early 1900s. An estimated 300,000 tons of calcium cyanamide is produced annually worldwide, with 80-85% of this amount used for fertilizer.

In 1993, the Health Effects Division RfD Committee selected a chronic dietary RfD of 0.002 mg/kg/day based on a long-term dog toxicity study (G. Ghali memo; *Hydrogen Cyanamide: RfD/Peer Review Report*; September 15, 1993). The HIARC chose not to revisit this RfD because RfDs are not required based on current and anticipated use patterns.

Calcium cyanamide is rapidly and quantitatively converted to hydrogen cyanamide at the low pH found in the gut. The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) determined that oral toxicity studies of the two chemicals can be used interchangeably. Hydrogen cyanamide was classified as **Group C–a possible human carcinogen** (W. Greear and E. Rinde memo; *Carcinogenicity Peer Review of Hydrogen Cyanamide*, September 5, 1993).

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Hydrogen cyanamide is one of several chemicals with metabolic properties similar to disulfiram (Antabuse®) which inhibit aldehyde dehydrogenase and are used to treat alcoholism. The HIARC considered the potential for Antabuse reactions in workers that consume ethanol–a consideration that was not evaluated in the animal studies.

Names: **Hydrogen cyanamide**, Cyanamide, amidocyanogen, carbamonitrile, carbimide, carbodimide, cyanoamine, N-cyanoamine, cyanogenamide, cyanogen nitride.

Structure:

$$H_2N–C=N$$

CAS #:	420-04-2
Empirical Formula:	CH_2N_2
Molecular Weight:	42.04 g/Mol
Melting Point:	45-46°C
Boiling Point:	83°C
Vapor Pressure:	5×10^{-3} Pa @ 20°C
Density (@ 20°C):	1.282
Solubility (water):	100 g/10 mL @ 43°C
Solubility (organic solvents):	Soluble in alcohols, phenols, amines, ethers, and ketones; very sparingly soluble in benzene and halogenated hydrocarbons; practically insoluble in cyclohexane.
Octanol/Water Partition Coefficient:	-0.82 @ 20°C
Reactivity:	Explosive polymerization may occur on evaporation of aqueous solutions to dryness.

2. HAZARD IDENTIFICATION

2.1 Acute Reference Dose (RfD)

An Acute Reference Dose is not required based on current and anticipated use patterns.

2.2 Chronic Reference Dose (RfD)

A Chronic Reference Dose is not required based on current and anticipated use patterns. The existing chronic RfD was not revisited since there are no food uses. If there is a need for a chronic RfD in the future, the HIARC recommends that the 1993 value (0.002 mg/kg/day) should be reconsidered.

2.3 Occupational/Residential Exposure

2.3.1 Short-Term (1-7 days) Incidental Oral Exposure

Based on current and anticipated use patterns, incidental oral endpoints are not required.

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2.3.2 Intermediate-Term (7 Days to Several Months) Incidental Oral Exposure

Based on current and anticipated use patterns, incidental oral endpoints are not required.

2.3.3 Dermal Absorption

Dermal Absorption Factor: 11%

Study Selected: Dermal Absorption in Rats · Guideline #: 870.7600

MRID No.: 41504004

Executive Summary: A single dermal dose of 0.1, 1, or 10 mg (8, 80, or 800 $\mu g/cm^2$, respectively) ^{14}C-hydrogen cyanamide, administered to three groups of male rats (24/group), was rapidly absorbed, distributed and eliminated. In general, as the dose level and the duration of exposure increased, the percentage of the dose absorbed also increased. Using the direct procedure to calculate skin absorption, the average ^{14}C-hydrogen cyanamide equivalents absorbed within 24 hours were 1.79%, 2.84%, and 11.1% of the applied dose for the low-, mid-, and high-dose groups, respectively.

Comments about Dermal Absorption: This study was classified Core Supplementary pending clarification of whether the material absorbed had unknown impurities (18-22%) present in the test material or pure hydrogen cyanamide. There is no record of these data being submitted. The HIARC determined that the results of this study can be used as a conservative measurement for risk assessment purposes.

2.3.4 Short-Term Dermal (1-7 days) Exposure

Study Selected: Developmental Toxicity in Rats Guideline #: 870.3700

MRID No.: 41288806

Executive Summary: In a developmental toxicity study in which rats were administered hydrogen cyanamide by gavage at dosages of 0, 10, 30, or 90 mg/kg/day (0, 5, 15, or 45 mg active ingredient/kg/day, respectively), maternal toxicity was observed at all dose levels tested. The LOAEL for maternal toxicity was less than or equal to 10 mg/kg/day [5 mg ai/kg/day], based on reductions in maternal body weight gain at all dose levels.

Developmental toxicity that included a nonsignificant increase in the number of resorptions, a significant reduction in fetal body weight, and significantly increased incidences of fetal anomalies was observed at the highest dose tested. The NOAEL and LOAEL for developmental toxicity were 30 and 90 mg/kg/day [15 and 45 mg ai/kg/day], respectively. The Developmental Toxicity Index (A/D Ratio; maternal LOAEL/developmental toxicity LOAEL) is 0.11, indicating that developmental toxicity was not observed below levels that produce maternal toxicity.

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Dose and Endpoint for Risk Assessment: Maternal LOAEL = 5 mg a.i./kg/day based on decreased body weight gain in dams.

Comments about Study/Endpoint: The target MOE is 300, which includes uncertainty factors (UFs) of 10 for interspecies extrapolation, 10 for intraspecies sensitivity, and 3 for use of a LOAEL. The endpoint selected does not reflect the potential for Antabuse effects in workers who concomitantly consume ethanol. The HIARC believes that the worker is better protected by a label warning to avoid ethanol consumption. Since an oral LOAEL was identified, an 11% dermal absorption factor should be used in route-to-route extrapolations.

2.3.5 Intermediate-Term Dermal (7 Days to Several Months) Exposure

Study Selected: Reproductive Toxicity in Rats Guideline #: 870.3800

MRID No.: 41566504

Executive Summary: In a two-generation reproduction study (MRID 41566504), 26 Crl:CD BR rats/sex/dose were administered aqueous hydrogen cyanamide (50% w/w) daily by gavage (10 mL/kg/day) at dose levels of 0, 2.5, 7.5, or 30 mg a.i./kg/day for the F_0 generation during premating; amended to 0, 1.25, 3.75, or 15 mg a.i./kg/day during week 12 and administered at these dose levels for the remaining generations: premating (F_1 generation), gestation, and lactation (F_0 and F_1 females). Exposure to P animals began at 7 weeks of age and lasted for 14 weeks prior to mating to produce F_1 pups. Upon weaning, F_1 pups (26/sex/dose) selected to become parents of the F_2 generation were administered the test chemical by gavage for 14 weeks prior to mating at the same concentrations their dams received. All animals were mated on a 1:1 ratio.

Statistically significant decreases in body weight, body weight gain, and food consumption were observed during the premating period in males and females in both generations. There was no compound-related effect on reproduction. There was a slight decrease in pup viability in the F_0 and F_1 pups, but the effect was not dose-related (the viability index in the control, low, mid, and high-dose pups was 92%, 83%, 88%, and 84% in the F_0 pups, and 93%, 87%, 82%, and 81% in the F_1 pups). There was also a slight decrease in F_0 and F_1 pup body weights in the dosed groups compared to controls, though the effect was not dose-related.

Parental NOAEL = 1.25 mg a.i./kg/day
Parental LOAEL = 3.75 mg a.i./kg/day (based on statistically significant decreases in body weight/weight gain and food consumption)
Reproductive NOAEL = 15 mg a.i./kg/day (highest dose tested)
Reproductive LOAEL >15 mg a.i./kg/day
Offspring NOAEL = Not determined
Offspring LOAEL = 1.25 mg a.i./kg/day (lowest dose tested; decreased pup viability and body weight in both generations)

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Dose and Endpoint for Risk Assessment: Offspring LOAEL = 1.25 mg a.i./kg/day based on decreased pup viability in the F1 and F2 generations. The decrease was seen in all three dose levels and was not dose-related. The LOAEL is the lowest dose tested, so there was no Offspring NOAEL in this study.

Comments about Study/Endpoint: Although the decrease in pup viability was not dose-related, the HIARC considered it to be a compound-related effect in both generations. The subchronic dog toxicity study, with a NOAEL of 1.0 mg a.i./kg/day and a LOAEL of 5.0 mg a.i./kg/day, was also considered when selecting an endpoint, but the rat offspring were considered to be more sensitive, and the impact on viability was considered the more severe effect. No paternal toxicity was observed at 1.25 mg/kg/day (the Parental NOAEL).

The target MOE is 300, which includes uncertainty factors (UFs) of 10 for interspecies extrapolation, 10 for intraspecies sensitivity, and 3 for use of a LOAEL. The endpoint selected does not reflect the potential for Antabuse effects in workers who concomitantly consume ethanol. The HIARC believes that the worker is better protected by a label warning to avoid ethanol consumption. Since an oral LOAEL was identified, an 11% dermal absorption factor should be used in route-to-route extrapolations.

2.3.6 Long-Term Dermal (Several Months to Life-Time) Exposure

Study Selected: Reproductive Toxicity in Rats Guideline #: 870.3800

MRID No.: 41566504

Executive Summary: See Intermediate-Term Dermal (7 Days to Several Months) Exposure.

Dose and Endpoint for Risk Assessment: Offspring LOAEL = 1.25 mg a.i./kg/day based on decreased pup viability in the F1 and F2 generations. The decrease was seen in all three dose levels and was not dose-related. The LOAEL is the lowest dose tested, so there was no Offspring NOAEL in this study.

Comments about Study/Endpoint: Although the decrease in pup viability was not dose-related, the HIARC considered it to be a compound-related effect in both generations. The subchronic dog toxicity study, with a NOAEL of 1.0 mg a.i./kg/day and a LOAEL of 5.0 mg a.i./kg/day, was also considered when selecting an endpoint, but the rat offspring were considered to be more sensitive, and the impact on viability was considered the more severe effect. No paternal toxicity was observed at 1.25 mg/kg/day (the Parental NOAEL).

The target MOE is 300, which includes uncertainty factors (UFs) of 10 for interspecies extrapolation, 10 for intraspecies sensitivity, and 3 for use of a LOAEL. The endpoint selected does not reflect the potential for Antabuse effects in workers who concomitantly

consume ethanol. The HIARC believes that the worker is better protected by a label warning to avoid ethanol consumption. Since an oral LOAEL was identified, an 11% dermal absorption factor should be used in route-to-route extrapolations.

2.3.7 Inhalation Exposure (All Exposure Durations)

Since hydrogen cyanamide is corrosive to the skin and eyes, it will also be highly irritating to the respiratory tract. The Merck Index (©1976) warns that, "Inhalation may cause irritation of mucous membranes." Hydrogen cyanamide is one of several chemicals with properties similar to disulfiram (Antabuse®) that are used clinically in the treatment of chronic alcoholism. Regulatory endpoints for inhalation must consider hydrogen cyanamide's potential to elicit respiratory irritation and Antabuse effects.

The only inhalation data available is an acute study which failed to report analytical concentrations (CIVO-CEN Study R4083; May 1, 1973; Accession No. 073726). The standing Toxicity Category of IV based on a nominal concentration is incorrect. The inhalation Toxicity Category has been changed to I by default due to corrosiveness.

Concentration and Endpoint for Risk Assessment: In 1991, OSHA selected an 8-hour time weighted average (TWA) threshold limit value (TLV) of 0.002 mg/L (2 mg/m^3) for hydrogen cyanamide. The TLV remains unchanged at this writing.[1] OSHA considers that this TLV is sufficiently low to prevent irritation of the respiratory mucosa, and is safely below a level that would produce an undesirable effect with ethanol ingestion.

Comments about Study/Endpoint: The HIARC chose to adopt OSHA's 8-hour TWA TLV–currently 0.002 mg/L–for calculating worker risk for short, intermediate, and long-term exposures because it is protective of hydrogen cyanamide's irritancy and Antabuse-effect. The Antabuse-effect of hydrogen cyanamide was assessed by comparing it to other chemicals used to treat alcoholism. The criteria used to select a non-irritating concentration were not specified.

Since the 0.002 mg/L TLV concentration is based on human data, the Target MOE would normally be 10 to account for intraspecies sensitivity. Since the sensitive population is known–workers that consume ethanol–and the TLV protects workers from the Antabuse-effect, there is no need to account for intraspecies sensitivity. Thus, the Target MOE is 1.

2.3.8 Margins of Exposure for Occupational/Residential Risk Assessments

There are no residential uses at the present time so MOEs are not required. For short, intermediate, and long-term occupational dermal exposure scenarios, the Target MOE is 300 which includes the conventional 100 and an additional 3x for the use of a LOAEL. For occupational inhalation exposure (any time period) a Target MOE of 1 is adequate

[1] Documentation of the Threshold Limit Values and Biological Exposure Indices, 6th Edition. ACGIH. Publication 0206. [MRID No. 45315601]

because a TLV, based on a human study, is used for risk assessments, and also would be protective of the sensitive population, i.e. workers who consume alcohol.

2.4 Recommendation for Aggregate Exposure Risk Assessments

An aggregate exposure risk assessment is not required since there are neither food uses nor residential uses at the present time. Also for occupational exposure, dermal and inhalation cannot be combined due to lack of a common toxicological endpoint via these routes.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

Carcinogenicity has been investigated in studies of hydrogen cyanamide and calcium cyanamide. Calcium cyanamide is rapidly and quantitatively converted to hydrogen cyanamide at the low pH found in the gut. The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) determined that oral carcinogenicity/toxicity studies of the two chemicals can be used interchangeably.

3.1 Combined Chronic Toxicity/Carcinogenicity Study in Rats - Calcium Cyanamide

MRID No.: None. Bioassay of calcium cyanamide. National Cancer Institute Study No. (NIH) 79-1719; issued in 1979.

Description of Bioassay: Groups of 50 male and 50 female F344 rats were dosed with calcium cyanamide in their feed at concentrations of 100 or 200 ppm (approximately 2.5 or 5.0 mg a.i./kg/day) for 107 weeks. An additional 20 males and 20 females served as negative controls.

This study was classified Core Supplementary. There were insufficient control animals. Dose concentration analyses were not performed. Body weights were not provided for months 15 through 18. Individual pathology sheets were not provided, so time-to-tumor data were not available and associations between tumor formation and deaths could not be made. The rats were housed in the same room as rats from two other bioassays (4-chloro-o-toluidine hydrochloride and N-nitrosodiphenylamine), and cross contamination could have occurred.

Discussion of Tumor Data: There was no statistically significant increase in tumor formation in any female dose group. There was a slight increase in the incidence of pheochromocytoma in HDT male rats; however, this increase was not statistically significant. Incidences were reported as 20%, 20%, and 32% in controls, 100, and 200 ppm groups, respectively. (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

Adequacy of the Dose Levels Tested: The dosing was considered adequate for assessing the carcinogenic potential of calcium cyanamide in this rat study, based upon the results of a range-finding study, and supported by the findings of a chronic dietary study with hydrogen cyanamide. (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

3.2 Carcinogenicity Study in Mice - Hydrogen Cyanamide

2-Year Drinking Water Carcinogenicity Study in Mice — Guideline #: 870.4200

MRID No.: 41566502, 42178405

Executive Summary: Hydrogen cyanamide was administered in the drinking water to groups of 60 CD-1 mice/sex for 100 weeks to males and for 104 weeks to females at levels of 0, 70, 200, or 600 ppm (for males: 0, 5.0, 13.6, and 36.8 mg a.i./kg/day; for females: 0, 6.9, 16.9, and 49.0 mg a.i./kg/day).

NOEL = 70 ppm (6.9 mg a.i./kg/day)
LEL = 200 ppm (16.9 mg a.i./kg/day) based on: females - increased incidence of urinary bladder and kidney lesions (non significant); decreased survival rate (Kaplan-Meier) in females.

In addition at 600 ppm in males (36.8 mg a.i./kg/day) there was decreased body weight gain (significant); in females (49.0 mg a.i./kg/day) the decreased survival was significant and there was ovarian stromal/luteal hyperplasia. In both sexes there were urinary bladder and kidney lesions.

Carcinogenic potential: Increased incidence (female) of ovarian granulosa-theca tumors and related tumors (thecoma, luteoma) at 200 and 600 ppm.

Discussion of Tumor Data: There were no significant compound-related tumors observed in male mice.

All of the ovarian tumors in female mice were combined according to the recommendation of Dr. Lucas Brennecke, HED's consulting pathologist; this combination was based on the definition provided by the study pathologist. It was determined that, for the statistical analysis, the ovarian granulosa theca tumors should be combined with the following tumor types considered borderline by the pathologist: luteinizing thecoma, granulosa-theca-luteal tumor, luteoma, thecal cell tumor, microscopic luteoma, and some granulosa-theca tumors.

For total ovarian granulosa-theca tumors there was a statistically significant increase ($p < 0.01$) by pair-wise comparison with controls at the HDT (600 ppm). There was also a statistically significant positive trend ($p < 0.01$).

The statistical analyses of tumor rates were based upon Peto's prevalence test since there was a statistically significant increasing trend in mortality with increasing doses of hydrogen cyanamide. To allow appropriate comparisons with historical control data, an additional analysis is provided which excludes the combined diagnoses. (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

Adequacy of the Dose Levels Tested: CPRC considered the dosing to be adequate for assessing the carcinogenic potential of hydrogen cyanamide. Adequacy of dosing was based on a significant increase in mortality in 600 ppm females (77%) compared to control (60%) and an increased

incidence of kidney and urinary bladder lesions in male and female mice at 600 ppm the highest dose tested (HDT). (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

3.3 Carcinogenicity Study in Mice - Calcium Cyanamide

MRID No.: None. Bioassay of calcium cyanamide. National Cancer Institute Study No. (NIH) 79-1719; Issued in 1979.

Description of Bioassay: Groups of 50 male and 50 female B6C3F1 mice were dosed with calcium cyanamide (estimated 48-66% a.i.) in their feed at concentrations of 500 or 2000 ppm (approximately 37 or 150 mg a.i./kg/day) for a period of 100 weeks. An additional 20 males and 20 females served as negative controls.

The study was classified Core Supplementary. Inadequate numbers of animals were used in the control groups. Food consumption, compound intake, and stability of test substance were not measured. Time-to-tumor formation data and individual pathology sheets were not provided; therefore, associations between tumor formation and deaths could not be established. In addition, the mice were housed in animal rooms in which seven other bioassays were in progress (1-chloroethyl trimethylammonium chloride; 2,4-diaminotoluene; lead dimethyldithiocarbamate; N-nitrosodiphenylamine; phthalamide; piperonyl sulfoxide; and 2,4,5-trimethylaniline) which could have provided the opportunity for cross-contamination.

Discussion of Tumor Data: The NCI report concluded that calcium cyanamide was negative for carcinogenicity in mice. However, two tumor types appeared to be increased in the treated mice in this study. The incidence of malignant hemangiosarcoma was increased in males at the HDT, and there was an increased incidence of malignant lymphoma in treated female mice.

There was a dose-related trend ($p < 0.01$) for hemangiosarcoma (all sites) in male mice; however, there was no statistically significant increase in this tumor type by pairwise comparison with the control group.

There was no increase in the incidence of hemangiosarcoma in female mice (9%, 0%, and 2% in control, 500 ppm, and 2000 ppm, respectively).

The historical control incidence of hemangiosarcomas in male B6C3F1 mice at the testing laboratory was 13/323 (4.0%). The highest incidence observed in any male control group at the testing laboratory was 2/19 (10.0%). The incidence of hemangiosarcoma in 2343 male B6C3F1 mice was been reported to range from 0% to 10% with a mean of 2.6% in studies conducted for NCI (Goodman, et al., In: Handbook of carcinogen Testing, 1985).

The NCI report indicated that tumors of the vascular system are relatively common in aging B6C3F1 mice. Although the incidence of hemangiosarcoma was twice the incidence observed in historical control mice, there was no increase in treated mice by pairwise comparison with control mice. However, the CPRC noted that part of the reason for the lack of a statistically significant increase in tumors by pairwise comparison with controls is the inadequate number of animals in

the control group of this study (n = 20) compared to the exposed group (n = 50). This may be illustrated if a hypothetical control group is created for the purposes of statistical testing, with the same rate of tumor formation as concurrent controls in this study (i.e. 5%). Comparison of the tumor incidence in this group with that of the 2000 ppm animal group results in a statistically significant increase in hemangiosarcoma ($p < 0.05$) by pairwise comparison with controls.

Although the NCI report concludes that the increased incidence of hemangiosarcomas in males at the HDT could not be attributed to the test material, the CPRC could not dismiss the tumors. Hemangiosarcoma is a malignant tumor, the rate of tumor formation at the HDT (20%) is very high, and it had a statistically significant positive trend. The CPRC believed that if an adequate number of control animals had been included, the pairwise comparison with controls may have been statistically significant as well. For these reasons, the CPRC considered the increase in hemangiosarcoma compound-related in male mice at the HDT.

The incidence of total lymphoma/leukemia was increased in females from the 500 ppm (24%) and 2000 ppm (36%) groups when compared to controls (5%). There was a statistically significant, dose-related trend ($p = 0.009$) and a statistically significant increase in the incidence of lymphoma/leukemias in the 2000 ppm group by pairwise comparison to control ($p = 0.006$).

The total incidence of lymphoma/leukemia at the HDT (36%) was within the upper end of the historical control range in 4/5 laboratories. The DER contained the suggestion that the low control incidence of lymphoma/leukemia made the incidence of this tumor type statistically significant that the HDT. The incidence of total lymphoma/leukemia in female control animals (5%) was below or equal to the lower end of the range of all five historical control studies.

At the time this study was conducted, one of the subclassifications of malignant lymphoma in the mouse was lymphoma, histiocytic. It is now believed that this type of neoplasm does not arise from the lymphoid series, but instead is a separate cell type altogether. Current classification of this neoplasm is histiocytic sarcoma. By removing "malignant lymphoma, histiocytic" from the list of lymphomas, the apparent treatment affect on lymphomas would certainly be lessened. However, there would then be an apparent significant treatment effect with regard to histiocytic sarcoma. Data from the NTP show that in 30 chronic (2-year oral) studies, the incidence of histiocytic sarcoma in female B6C3F1 mice was 13/1470 (0.88%), with a range of 0% to 4%. Obviously, since the neoplasm was classified as a lymphoma at the time the study was conducted, no contemporary historical data were available on histiocytic sarcoma in the B6C3F1 mouse. Under the current classification, the incidence of that tumor would far exceed the range of historical controls (L. Brennecke, Pathology Associates Inc.).

Given these limitations and for reasons described above, it was difficult for the CPRC to determine the biological significance of lymphoma/leukemia in female mice. Therefore, the CPRC agreed to accept the conclusions of the NCI. (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

Adequacy of the Dose Levels Tested: Dosing was considered adequate for assessing the carcinogenic potential of calcium cyanamide in this mouse study, based upon the results of the range-finding study. (Carcinogenicity Peer Review of Hydrogen Cyanamide; September 15, 1993)

3.4 Classification of Carcinogenic Potential

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on September 1, 1993 to discuss and evaluate the weight-of-the evidence on hydrogen cyanamide with particular reference to its carcinogenic potential. The CPRC concluded that hydrogen cyanamide should be classified as **Group C–possible human carcinogen**, and recommended that for the purpose of risk characterization a low dose extrapolation model applied to the experimental animal tumor data should be used for quantification of human risk Q_1*. The CPRC agreed that the Q_1* should be based on ovarian (total granulosa-theca) tumors observed in female CD-1 mice in the drinking water study.

The decision was based on the statistically significant increase in the incidence of ovarian granulosa-theca tumors in female CD-1 mice both by positive trend and pairwise comparison with controls at the HDT, the positive trend in hemangiosarcomas in male B6C3F1 mice, and the activity in two mutagenicity assay systems. The decision of the CPRC was recorded in a memorandum drafted by William Greear and Esther Rinde, and dated September 15, 1993.

Based upon female mouse ovarian granulosa-theca tumor rates in a 104-week drinking water study, the unit risk, Q_1^* $(mg/kg/day)^{-1}$, for hydrogen cyanamide is 6.64×10^{-2} in human equivalents using a mg/kg body weight$^{3/4}$/day interspecies scaling factor (Lori L. Brunsman memorandum; March 22, 2001). There was no evidence of carcinogenicity in rats, mice, or dogs in other long-term studies.

4 MUTAGENICITY

4.1 Ames Assay

Eight doses of hydrogen cyanamide ranging from 0.10 to 15.0 µL/plate (or 0.02 to 2.54 mg/plate) failed to increase the number of revertant colonies in *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, and TA-100 in repeat assays with and without the presence of a metabolic activation system. The HDT produced slight toxicity, as evidenced by reduced numbers of revertants in all the strains tested. [MRID No. 40389608]

4.2 In Vitro Cytogenetics (CHO Assay)

The test material induced dose-related chromosomal aberrations in Chinese hamster ovary cells into the toxic range under conditions of nonactivation and metabolic activation. [MRID No. 40389609]

4.3 Micronucleus Test

The test material failed to induce significant increases in micronuclei in bone marrow polychromatic erythrocytes over a dose range from 31.44 to 330.5 mg/kg, the HDT inducing clinical toxicity (death and ruffled coats). [MRID No. 40389610]

4.4 Unscheduled DNA Synthesis in Primary Rat Hepatocytes

The test material did not induce significant changes in nuclear labeling of primary hepatocytes over a dose range of 5.95 to 143 μg/mL when compared to the solvent control. [MRID No. 40389607]

5 FQPA CONSIDERATIONS

An FQPA assessment is not required because there are no tolerances, no residential exposures, and no expectations of a change in the current use pattern.

6 HAZARD CHARACTERIZATION

Toxicity Data Base Overview: Based on hydrogen cyanamide's "non-food" use pattern, the HIARC has determined that there are no data gaps. Oral toxicity is well characterized. Other than acute studies which cannot provide regulatory endpoints, there are no dermal or inhalation studies, and none are required due to the corrosive nature of hydrogen cyanamide.

Acute Toxicity: The Toxicity Categories are **II** for oral toxicity, dermal toxicity, and eye irritation, and **I** for skin irritation. Hydrogen cyanamide is a strong dermal sensitizer, and is corrosive to the skin up to 7 days after dosing. All treated rabbit eyes had slight corneal opacity, mild iritis, moderate redness, and moderate to severe conjunctival swelling after 24 hours with slight conjunctivitis on day 7. Since hydrogen cyanamide is corrosive, and the Merck Index (©1976) states that, "Inhalation may cause irritation of mucous membranes," the Toxicity Category for inhalation is **I** by default.

Route-Specific Toxicity: Hydrogen cyanamide was found to be a strong dermal sensitizer in guinea pigs. In a primary skin irritation study, corrosivity in rabbits persisted to the end of the study (day 7). Also, severe eye irritation was seen in rabbits. Considering that the respiratory mucosa is far more sensitive to chemical insult than the skin, and at least as sensitive as the mucous membranes of the eye, it ◆> is reasonable to assume that serious respiratory trauma could result if humans inhale hydrogen cyanamide. Because there are no route-specific dermal or inhalation endpoints, dermal risk assessments are based on oral endpoints extrapolated using an estimated dermal absorption factor, and inhalation risk assessments are based on an OSHA TLV.

Pharmacokinetics: A dermal penetration study (MRID No. 41504004) demonstrated that hydrogen cyanamide is rapidly absorbed, distributed and eliminated. In general, as the dose level and the duration of exposure increased, the percentage of the dose absorbed also increased. At dermal doses of 0.1, 1, or

10 mg (8, 80, or 800 $\mu g/cm^2$, respectively) the average ^{14}C-hydrogen cyanamide equivalents absorbed within 24 hours were 1.79%, 2.84%, and 11.1%.

Two metabolism studies were performed. The first study (MRID No. 42178407) showed that hydrogen cyanamide is rapidly absorbed, metabolized, and excreted in the urine following oral, intravenous (IV), and intraperitoneal (IP) dosing in rats, dogs, or rabbits. Following IP dosing in rats, 93.4% of the dose was excreted in the urine within the first 6 hours, indicating that hydrogen cyanamide is rapidly metabolized and almost completely eliminated from the body. Negligible amounts were excreted as expired CO_2. Following both oral and IV dosing in dogs, 62 - 83.1% of the dose was excreted in the urine within the first 24-27 hours. Negligible amounts of radioactivity were excreted in the feces. The major metabolite of cyanamide excreted in the urine of rats, rabbits, dogs, and humans was identified as N-acetylcyanamide. The conversion of cyanamide to N-acetylcyanamide *in vitro* is catalyzed by an acetyl-S-CoA-dependent N-acetyltransferase present in rabbit and dog liver.

The second metabolism study (MRID No. 42906401) demonstrated that ^{14}C-hydrogen cyanamide is rapidly absorbed, distributed, metabolized, and excreted following oral and IV administration in rats. Total radioactivity recoveries were high for all oral and IV groups (98.7-105.5% of administered dose). Urine was the major route of excretion (66.9-97.7% of administered dose). Hydrogen cyanamide elimination was sex-, dose-, and route-related.

Cumulative Toxicity: A comparison of acute, subchronic, and chronic studies shows a marked cumulative response over the subchronic time frame, but possibly an adaptive response thereafter.

Subacute and Subchronic Toxicity: A 21-day dermal toxicity study was not performed. In an unacceptable subchronic feeding toxicity study in rats (Accession No. 073726), the NOAEL is 0.5 mg a.i./kg/day based on morphological activation observed in the thyroids of males at the LOAEL of 1.5 mg a.i./kg/day consisting of an increased number of small follicles lined by cuboidal epithelium and disappearance of colloid. The number of interfollicular cells was increased and occasionally accompanied by a marked proliferation of follicular epithelial cells.

In a subchronic feeding toxicity study in dogs (MRID No. 413000501), oral dosing was traumatic to the buccal cavity as evidenced by heavy salivation prior to dosing, struggled during dosing, redness of the buccal mucosa, and the presence of inflammatory cells in the parotid salivary glands. Dose-related effects, which were observed mostly at the LOAEL of 6 mg a.i./kg/day include a decrease in female body weights, decreased absolute and relative testicular weights (males) and thymic weights (females), testicular atrophy, and impaired spermatogenesis. The NOAEL was 2 mg a.i./kg/day.

Developmental and Reproductive Toxicity: In a rat gavage developmental toxicity study (MRID No. 41288806), the maternal and developmental NOAELs were <5 mg a.i./kg/day and 15 mg a.i./kg/day, respectively. An increase in the number of resorptions, decreased fetal body weight, increase in diaphragmatic hernias, and wavy/bent ribs were seen at the developmental LOAEL of 45 mg a.i./kg/day, which is significantly greater than the maternal LOAEL of 5 mg a.i./kg/day. The only maternal effect seen at the LOAEL was decreased body weight gain.

There were two gavage developmental toxicity studies in rabbits. In the first (Accession No. 073727), the maternal and developmental NOAELs were 2 mg a.i./kg/day and 6 mg a.i./kg/day, respectively. There was an increase in early resorptions and dead fetuses, a decrease in fetal weight and size, an increase in small meningeal hemorrhages and/or hemorrhages of the olfactory bulb, and disintegration of liver structure at the LOAEL of 18 mg a.i./kg/day. The maternal LOAEL was 6 mg a.i./kg/day based on a decrease in body weight gain.

In the second rabbit study (MRID Nos. 41288805 and 42178406), the maternal and developmental NOAELs were both 6 mg a.i./kg/day. The maternal LOAEL was 18 mg a.i./kg/day based on decreased body weight gain, and the developmental LOAEL was 18 mg a.i./kg/day based on disintegration of liver structure and a slight decrease in female size and weight.

A gavage reproductive toxicity study was performed in rats (MRID No. 41566504). The parental NOAEL was 1.25 mg a.i./kg/day based on statistically significant decreases in body weight, body weight gain, and food consumption at the paternal LOAEL of 3.75 mg a.i./kg/day. The reproductive NOAEL was 15 mg a.i./kg/day, the highest dose tested. The HIARC determined that an offspring NOAEL could not be determined because of slight decreases in viability and body weights in the F_0 and F_1 pups, though these effects were not dose-related (the viability index in the control, low, mid, and high-dose pups was 92%, 83%, 88%, and 84% in the F_0 pups, and 93%, 87%, 82%, and 81% in the F_1 pups).

Chronic Toxicity/Carcinogenicity and Mutagenicity: In a chronic dog study (MRID Nos. 41288802 and 41566501), there were increased incidences of rough haircoat, desquamation of the skin, tremors, and salivation; decreased body weight gain; decreased T_4 in males; increased relative thyroid-parathyroid weights in females; brown pigment in liver Kupffer cells; thymic atrophy in males; testicular inflammation; and aspermatogenesis, and hypospermatogenesis at the LOAEL of 5.0 mg a.i./kg/day. The NOAEL in this study was 1.0 mg a.i./kg/day. There were no neoplastic lesions.

Carcinogenicity has been investigated in studies of hydrogen cyanamide and calcium cyanamide. Because calcium cyanamide is rapidly and quantitatively converted to hydrogen cyanamide at the low pH found in the gut, the Health Effects Division Carcinogenicity Peer Review Committee (CPRC) determined that oral carcinogenicity/toxicity studies of the two chemicals can be used interchangeably.

There was no evidence of carcinogenicity in a chronic rat study of hydrogen cyanamide (MRID No. 42178404). The NOAEL was 2.5 mg a.i./kg/day based on significant decreases in body weight gain, reduced colloid in the thyroid, and reduced T_3 and T_4 levels, and decreased erythrocyte count, hemoglobin, and hematocrit at the LOAEL of 7.5 mg a.i./kg/day.

National Cancer Institute bioassays of calcium cyanamide in rats and mice (Accession No. 073727) were negative for carcinogenicity. The CPRC determined that dosing was adequate for assessing the carcinogenic potential of calcium cyanamide in both studies, based upon the results of range-finding studies and, in the case of the rat study, support of the chronic dietary study with hydrogen cyanamide.

The only study that was positive for carcinogenicity was a mouse carcinogenicity study of hydrogen cyanamide in drinking water (MRID Nos. 41566502 and 42178405). Females had an increased incidence of urinary bladder and kidney lesions and a decreased Kaplan-Meier survival rate at the LOAEL of 16.9

mg a.i./kg/day. At 38.8/49.9 mg a.i./kg/day (M/F), there was decreased body weight gain in males, decreased survival, ovarian stromal/luteal hyperplasia in females, and urinary bladder and kidney lesions in both sexes. There was an increased incidence of ovarian granulosa-theca tumors and related tumors (thecoma, luteoma) at 200 and 600 ppm. These findings were used by the Health Effects Division Carcinogenicity Peer Review Committee (CPRC) to classify hydrogen cyanamide as Group C - possible human carcinogen. Based upon female mouse ovarian granulosa-theca tumor rates in a 104-week drinking water study, the unit risk, Q_1^* $(mg/kg/day)^{-1}$, for hydrogen cyanamide is 6.64×10^{-2} in human equivalents using a mg/kg body $weight^{3/4}$/day interspecies scaling factor (Lori L. Brunsman memorandum; March 22, 2001).

In an *in vitro* cytogenetics assay, the test material induced dose-related chromosomal aberrations in Chinese hamster ovary cells at toxic levels under conditions of nonactivation and metabolic activation [MRID No. 40389609]. There was no evidence of mutagenicity in an Ames assay (MRID No. 40389608), *in vivo* micronucleus assay in mice (MRID No. 40389610), or an unscheduled DNA synthesis in rat hepatocytes (MRID No. 40389607).

Neurotoxicity: No neurotoxicity studies were conducted. The only suggestion of neurotoxicity in the entire data base was tremors and excessive salivation in the chronic dog toxicity study at the LOAEL of 5 mg a.i./kg/day. Neurotoxicity is mentioned in the literature, however. Grant (1986)[2] reports that acutely poisoned animals show signs of parasympathetic overactivity including miosis, excessive salivation, tearing, and twitching.

Endocrinopathy: None of the animal studies provide any evidence that hydrogen cyanamide disrupts endocrine receptors.

7 DATA GAPS

Based on the current use pattern, there are no data gaps. Although the acute inhalation toxicity study is inadequate, a new study is not required because the chemical classified Toxicity Category I by default due to corrosivity. The 90-day inhalation toxicity study and the dermal toxicity study are waived due to corrosivity.

[2] Grant, W.M. Toxicology of the eye. Third edition. Springfield, Illinois; Charles C. Thomas Publisher. Page 286. 1986.

8 ACUTE TOXICITY

Acute Toxicity of Hydrogen Cyanamide

Guideline No.	Study Type	MRIDs	Results	Toxicity Category
870.1100	Acute Oral	Accession 073726	$LD_{50} \approx 300$ mg a.i./kg	II
870-1200	Acute Dermal	41288801	$LD_{50} = 1700$ mg a.i./kg ♂ $LD_{50} = 1400$ mg a.i./kg ♀	II
870.1300	Acute Inhalation	Accession 073726	The standing Toxicity Category of IV is erroneous because it is based on a nominal concentration rather than an analytical concentration. Because hydrogen cyanamide is corrosive to the skin, eyes, and mucous membranes, the Toxicity Category is changed to a default of I.	I
870.2400	Primary Eye Irritation	Accession 073726	Slight corneal opacity, mild iritis, moderate redness, and moderate to severe swelling of the conjunctiva in all rabbits after 24 hours. All rabbits had slight conjunctivitis on day 7.	II
870.2500	Primary Skin Irritation	NA	Corrosive up to 7 days (end of test)	I
870.2600	Dermal Sensitization	Accession 073726	Strong sensitizer	–
870.6200	Acute Neurotoxicity	–	–	–

9. SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg a.i./kg/day)	ENDPOINT	STUDY
Acute Dietary	Reference Doses (RfDs) are not required based on current and anticipated use patterns.		
Chronic Dietary			
Incidental Oral, Short-Term	Incidental oral endpoints are not required based on current and anticipated use patterns.		
Incidental Oral, Intermediate-Term			
Dermal, Short-Term[a]	LOAEL = 5	Decreased dam body weight gain. Dermal absorption = 11%. **Target MOE = 300**	Developmental toxicity in rats
Dermal, Intermediate-Term[a]	LOAEL = 1.25	Decreased F1 and F2 pup viability. Dermal absorption = 11%. **Target MOE = 300**	Reproductive toxicity in rats
Dermal, Long-Term[a]	LOAEL = 1.25	Decreased F1 and F2 pup viability. Dermal absorption = 11%. **Target MOE = 300**	Reproductive toxicity in rats
Inhalation, (All Exposure Durations)	0.002 mg/L	Irritation of the respiratory mucosa and Antabuse® response in workers who consume ethanol. **Target MOE = 1**	OSHA 8-h TWA TLV (human data)

[a] Since an oral LOAEL was identified, a 11% dermal absorption factor should be used in route-to-route extrapolations.

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