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

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MEMORANDUM

15 SEP 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Carcinogenicity Peer Review of Hydrogen Cyanamide

FROM: William Greear, M.P.H. and William B. Greear 9/10/93
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Health Effects Division (H7509C)

and

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TO: Joanne I. Miller
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THROUGH: Penelope A. Fenner-Crisp 9/13/93
Penelope Fenner-Crisp, Ph.D.
Director, Health Effects Division (H7509C)

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.

This 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.

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A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Reto Engler

William Burnam

Karl Baetcke

Kerry Dearfield

Elizabeth Doyle

Esther Rinde

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Marion Copley[1]

Lori Brunsman

For Bernice Fisher

Lucas Brennecke[2]
(PAI/Clement)

3. Other Attendees:

Nan Gray (HED)

Diane Mandell (Clement)

[1]Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

[2]Signature indicates concurrence with pathology report.

B. Material Reviewed:

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The material available for review consisted of DERs and other data summaries prepared by William B. Greear and Marion Copley, and statistical analyses prepared by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Hydrogen Cyanamide is a plant growth regulator which has been approved for use on grapes under several Section 18 Emergency Exemptions. It has properties similar to disulfiram (Antabuse®) which is used to treat chronic alcoholism. The following synonyms pertain to Hydrogen Cyanamide: Cyanamide, Carbimide, Carbodiimide, Dormex®, Amidocyanogen, Cyanogenamide, SKW 83010 and Alzodef®.

Calcium Cyanamide is rapidly and quantitatively converted to Hydrogen Cyanamide in solution and at the pH of human and rodent gut. Thus, oral carcinogenicity/toxicity tests conducted with Calcium Cyanamide are interchangeable with those conducted with Hydrogen Cyanamide, and were therefore used as supporting data for this chemical.

The current Caswell (or Tox Chem) Number of Hydrogen Cyanamide is 140. The current Caswell Number of Calcium Cyanamide is 140A[3]. The Chemical Abstract Registry Number (CAS No.) of Hydrogen Cyanamide is 420-04-2, and the CAS No. of Calcium Cyanamide is 156-62-7. The PC Code No. of Hydrogen Cyanamide is 014002, and the PC Code No. of Calcium Cyanamide is 014001.

The structure of Hydrogen Cyanamide is presented below:

$$H_2NC{\equiv}N$$

D. Evaluation of Carcinogenicity Data

1. Mouse 2-Year Drinking Water Carcinogenicity Study

Reference: Hydrogen Cyanamide oncogenicity drinking water study in mice. MRID No. 415665-02, Study No. 6001-556/3. Testing Facility: Hazleton UK, North Yorkshire, England. Study dated May 3, 1990.

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[3]There is some confusion in the one-liners as to the Caswell No. for these substances; in some cases they have been used interchangeably. Outdated Caswell Numbers for Hydrogen Cyanamide are 485A and 266B; the new updated Caswell Numbers supersede these.

a. Experimental Design

Technical Hydrogen Cyanamide (approximately 50% a.i.) was administered in drinking water to groups of 60 male and 60 female CRL:CD-1(ICR)BR mice at doses of 0, 70, 200 or 600 ppm (equivalent in males to 0, 9.9, 27.1, or 73.6 mg/kg/day test compound or 0, 5.0, 13.6, or 36.8 mg/kg/day a.i.; equivalent in females to 0, 13.8, 33.8 or 98.0 mg/kg/day test compound or 0, 6.9, 16.9, or 49 mg/kg/day a.i.) for a period of 100 weeks (males) or 104 weeks (females).

b. Discussion of Tumor Data

There were no significant compound-related tumors observed in male mice.

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: luteinized 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 ($p < 0.01$, Table 1).

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.

Table 1. Hydrogen Cyanamide - Charles River CD-1 Mouse Study

Female Ovarian Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	70	200	600
TOTAL TUMORS[a]				
Granulosa-Theca Tumors	3/59	1/59	7[b]/57	13/56
(%)	(5)	(2)	(12)	(23)
p =	0.000**	-	0.116	0.001**
GRANULOSA-THECA TUMORS ONLY FOR COMPARISON WITH HISTORICAL CONTROL DATA				
Granulosa-Theca Tumors	3/51	1/47	6/45	8[c]/42
(%)	(6)	(2)	(13)	(19)
p =	0.008**	-	0.166	0.057

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[a]Includes granulosa-theca tumors along with the following tumor types considered borderline by the pathologist: luteinized thecoma, granulosa-theca-luteal tumor, luteoma, thecal cell tumor, microscopic luteoma, and some granulosa-theca tumors.

[b]First "borderline" granulosa-theca tumor observed at week 29, dose 200 ppm.

[c]First granulosa-theca tumor observed at week 78, dose 600 ppm.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The weighted average of ovarian granulosa-theca tumors in female historical control CRL:CD-1(ICR)BR mice in 104-week studies was 1.5%, with a range of 0 to 6% (Table 2). The incidence of ovarian granulosa-theca tumors in the 600 ppm group (19%) was well above the upper end of the historical control range observed in all 14 studies. In 7/14 studies, the tumor incidence was 0.

Table 2. Historical Controls: 104-Week Studies. Incidence of Granulosa-Theca (Ovarian) Tumors in Female CRL:CD-1(ICR)BR mice[a]

Study No.	Incidence (%)
1	3/60 (5)
2	0/60 (0)
3	1/90 (1)
4	2/49 (4)
5	0/51 (0)
6	0/51 (0)
7	3/51 (6)
8	0/51 (0)
9	0/51 (0)
10	1/62 (2)
11	1/51 (2)
12	0/51 (0)
13	0/51 (0)
14	1/50 (2)
Weighted average	(1.5)

[a] Historical data for granulosa-theca ovarian tumors were obtained from 14 chronic/carcinogenicity studies with female CRL:CD-1(ICR)BR mice started at Hazleton UK between 1981 and 1990. Studies were 104 weeks in duration. Data were provided by the registrant after the initial study was submitted.

c. Non-neoplastic lesions and other findings

There was no differential mortality in male mice. The female mice showed a statistically significant increasing trend in mortality with increasing doses of Hydrogen Cyanamide. Females in the control, 70, 200, and 600 ppm groups had cumulative mortality rates of 60, 66, 79, and 77%. Mortality was significantly increased in 600 ppm females by pairwise comparison with controls. The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Body weight in male mice was significantly reduced from controls in HDT males after 52 weeks on study; however body weight gain was not significantly different from controls at study termination. Females experienced early decreases in body

weight gain; however, from 6 weeks on test until study termination, female mice were not significantly different from control mice.

There was an increase in kidney lesions in male and female mice (Table 3). At 600 ppm, males exhibited increased incidence of fibrosis/scarring, atrophic/basophilic tubules, granular casts and vacuolar degeneration/necrosis compared to control mice. At 200 ppm males exhibited an increase in vacuolar degeneration/necrosis. At 600 ppm, females exhibited increases in fibrosis/scarring, tubular dilation, granular casts and vacuolar degeneration/necrosis. Chronic cystitis of the urinary bladder was increased in males in the 200 and 600 ppm groups when compared to controls. Chronic cystitis was increased in females in the 200 and 600 ppm groups when compared to controls.

Table 3. Incidence (%) of Non-Neoplastic Lesions in CD-1 (ICR) BR Mice[a]

	Levels in Drinking Water (ppm)							
Lesions	Males				Females			
	0	70	200	600	0	70	200	600
Kidney, n=	59	59	59	58	60	60	59	58
fibrosis/ scarring	1 (1.7)	1 (1.7)	1 (1.7)	7 (12.1)	0 (0)	1 (1.7)	1 (1.7)	6 (10.3)
atrophic/ baso- phili c tubules	28 (47.5)	28 (47.5)	29 (49.2)	36 (62.1)	20 (33.3)	19 (31.7)	15 (25.4)	25 (41.7)
tubular dilation	8 (13.6)	11 (18.6)	12 (20.3)	8 (13.8)	8 (13.3)	6 (10.0)	9 (15.3)	15 (25.9)
granular casts	1 (1.7)	0 (0)	2 (3.4)	5 (10.3)	1 (1.7)	0 (0)	2 (3.4)	5 (8.6)
vacuolar degener- ation/ necrosis	0 (0)	1 (1.7)	5 (8.5)	12 (8.5)	3 (5.0)	1 (1.7)	5 (8.5)	9 (15.5)

[a]Data were abstracted from Study No. 6001-556/3, Table 8.7.

There was a marginal increase in ovarian stromal/luteal hyperplasia in females in the 600 ppm group when compared to controls. The CPRC noted that the ovarian lesions may have been related to the formation of tumors in this tissue.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The 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).

2. NCI Mouse Study, Two-Year Carcinogenicity Bioassay with Calcium Cyanamide

Reference: Bioassay of Calcium Cyanamide for possible carcinogenicity. National Cancer Institute Study No. (NIH)79-1719. Testing Facility: NCI Frederick Cancer Research Center, Frederick, MD. Report issued in 1979.

Data and tables were extracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 005681 [DER for the National Toxicology Program (NTP) Mouse Oncogenicity Study].

a. Experimental Design

Calcium Cyanamide (estimated 48-66% a.i.) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice at dosages of 0, 500 or 2000 ppm (approximately 37 or 150 mg/kg/day a.i.) for a period of 100 weeks. The control group consisted of 20 mice/sex.

This study suffered from several flaws. 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, which could have provided the opportunity for cross-contamination.

b. 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.

The incidence of hemangiosarcoma in male mice is shown in Table 4. 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 (0%, 0%, and 2% in control, 500 ppm, and 2000 ppm, respectively).

Table 4. Calcium Cyanamide - NTP Study

Male Hemangiosarcoma Rates in B6C3F1 Mice[a]

	Dose (ppm)		
	0	500	2000
Hemangiosarcoma (%)	1/20 (5)	2/50 (4)	10/50 (20)
p =	< 0.01	-	0.116

Note: Significance of trend denoted at control (Cochran-Armitage test).

[a] Abstracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 005681 (DER for the NTP Mouse Oncogenicity Study).

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 has 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 concluded 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.

Table 5 shows the incidence of total lymphoma/leukemias (including all lymphoma variations, lymphocytic leukemia and monocytic leukemia) in female mice. 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 ($p = 0.006$).

Table 5. Calcium Cyanamide - NTP Study

Female Malignant Lymphoma and Leukemia Rates in B6C3F1 Mice[a]

	Dose (ppm)		
	0	500	2000
Malignant Lymphoma, NOS[b]	0/20	1/46	3/50[c]
(%)	(0)	(2)	(6)
Malignant Lymphoma, histiocytic	1/20	9/46	12/50[c]
(%)	(5)	(19)	(24)
Malignant Lymphoma, lymphocytic	0/20	0/46	1/50
(%)	(0)	(0)	(2)
Malignant Lymphoma, mixed	0/20	0/46	0/50
(%)	(0)	(0)	(0)
Lymphocytic Leukemia	0/20	1/46	0/50
(%)	(0)	(2)	(0)
Monocytic Leukemia	0/20	0/46	2/50
(%)	(0)	(0)	(4)
Total	1/20	11/46[d]	18/50
(%)	(5)	(24)	(36)
p =	0.009**	-	0.006**

[a] Table extracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 005681 (DER for the NTP Mouse Oncogenicity Study).

[b] Not Otherwise Stated

[c] 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.

[d] 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.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

The historical control incidence of lymphoma/leukemia in B6C3F1 mice has been reported for five laboratories conducting studies for NCI (Tarone et al., JNCI 66(6): 1175-81, 1981) (Table 6).

Table 6. Historical Controls[a]

Incidence of Lymphoma/Leukemia in Female B6C3F1 Mice

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

[a] Table extracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 005681 (DER for the NTP Mouse Oncogenicity Study).

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 at 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 was 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.

c. Non-neoplastic Lesions and Other Findings

Mortality was decreased in the HDT males only. Mortality in this group was 24% compared to 0% in control males. Mortality in females was not affected by treatment with the test substance.

Body weight was "slightly decreased" in both male treatment groups, and in females at the HDT (estimated from graphs, no tabular data was available). It was difficult to evaluate the magnitude of this decrease, since the report contained no tables of animal body weights.

Gross and histopathological examinations were performed, and were stated to be "unremarkable."

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

The 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.

A seven-week subchronic feeding study in mice was initially conducted in order to establish adequate dosing. Groups of 5 mice/sex were administered 0, 1500, 3000, 4000, 8000, 10,000, 16,000 or 30,000 ppm dietary Calcium Cyanamide. Body weight gain was decreased at all dose levels. No deaths occurred at doses ≤ 16,000 ppm, while mortality was 100% at 30,000 ppm. Mild bile-duct hyperplasia was observed in male and female mice in the 16,000 ppm dose group. Periportal hepatocytes with pale-staining vacuolated cytoplasm was seen in male mice. Focal hepatic necrosis was present in 4/5 female mice. Body weight was decreased 7-8% and 13-15% in males and females, respectively, in the 1500, 3000 and 4000 ppm groups. Ten percent body weight depression was a major criterion for estimation of the doses in mice using least squares regression of probit analysis.

3. NCI Rat Study, Two-Year Carcinogenicity Bioassay with Calcium Cyanamide

Reference: Bioassay of Calcium Cyanamide for possible carcinogenicity. National Cancer Institute Study No. (NIH)79-1719. Testing Facility: NCI Frederick Cancer Research Center, Frederick, MD. Report issued in 1979.

Data and tables were extracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 005681 (DER for the NTP Rat Oncogenicity Study).

a. Experimental Design

Calcium Cyanamide (48-66% a.i. estimated) was administered in the diet to groups of 50 male and 50 female F344 rats at

dosages of 0, 100 or 200 ppm in males (approximately 2.5 or 5 mg/kg/day a.i.), and 0, 100 or 400 ppm in females (approximately 2.5 or 10 mg/kg/day a.i.) for a period of 107 weeks. The control group consisted of 20 rats/sex.

As mentioned with regard to the mouse study, this study also suffered from several flaws. 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 rats were housed in animal rooms in which two other bioassays were in progress, which could have provided the opportunity for cross-contamination.

b. 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.

c. Non-Neoplastic Lesions

Survival was unaffected by compound administration. Mean body weights of male and female rats at the HDT was "slightly lower" than controls (estimated from graphs, no tabular data was available). It was difficult to evaluate the magnitude of this decrease, since the report contained no tables of animal body weights.

There was an increased incidence of dilation of the ducts and hyperplasia of the mammary gland in males at the HDT (200 ppm). There was also an increase in the incidence of hyperplasia (only) of the mammary gland in males at 100 ppm.

d. Adequacy of Dosing for Assessment of Carcinogenic Potential

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 (discussed below), and supported by the findings of a chronic dietary study with Hydrogen Cyanamide (see Section E.3).

Two 7-week subchronic feeding studies were initially conducted in order to estimate adequate dose levels. In one study, 5 rats/sex/group were administered 0, 1500, 3000, 4000, 8000, 10,000, 16,000 or 30,000 ppm of Calcium Cyanamide in the diet. The second study tested 5 rats/sex/group at 0, 400, 600, 800, 900, 1000, 1200 or 1500 ppm. Body weight gain was decreased

at all levels and 100% mortality was observed at dose levels above 400 ppm. Trace-to-moderate amounts of bile duct hyperplasia were observed in rats dosed with 1500, 3000 and 4000 ppm. Slight-to-moderate increase in extramedullary hematopoiesis was observed in the spleen of males and females (doses not specified). Marked diffuse hyperplasia of the thyroid was observed at the 4000 ppm dose level. Thyroid hyperplasia was also observed in rats in the 400-3000 ppm groups.

E. Additional Toxicological Data on Hydrogen Cyanamide

1. Metabolism

Reference: Metabolism of Hydrogen Cyanamide, SKW Trostberg AG, FRG, Study Completed September 5, 1991. MRID No. 421784-07.

In solution (in vitro), and in the human and rodent gut, Calcium Cyanamide is rapidly converted to Hydrogen Cyanamide. Toxicity studies can be used interchangeably for either compound for toxicity endpoints.

Hydrogen Cyanamide is rapidly absorbed, metabolized and excreted in the urine following oral, intravenous (i.v.), and intraperitoneal (i.p.) dosing in rats, dogs, or rabbits. Following i.p. 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 carbon dioxide. Following both oral and i.v. dosing in dogs, 62%-83% 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, rabbit, 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.

2. Genotoxicity

Reference: MRID Nos. 403896-07, 403896-08, 403896-09, 403896-10

Hydrogen Cyanamide and Calcium Cyanamide have been tested in several mutagenicity assay systems (Table 7). In a positive in vitro cytogenetics (CHO) study, the response was twice that of the positive controls with and without metabolic activation and a dose-response relationship was seen. In addition, an Ames assay performed under the auspices of the NTP revealed weak positive results in Salmonella strain TA1535 in two laboratories. In a submitted study, negative results were obtained with the same strain (and others). Acceptable tests fulfill all 3 categories for mutagenicity testing. The following studies are available:

Table 7. Summary of Genotoxicity Studies for Hydrogen/Calcium Cyanamide.

Study	Results	Study Status
Gene mutation Ames Test (weak positive in Salmonella strain TA1535 at ≥ 333 μg/plate with rat or hamster S9; negative without activation. Results consistent from two laboratories testing for NTP (CaCN).	**Weak Positive (+ activ.)**	Published study[4]
Sex-linked Recessive Lethal Study in Drosophila (CaCN).	Negative	Published study[5]
Ames Test (Salmonella negative in strains TA1535, TA1537, TA1538, TA98 and TA100). Tested at up to 15 μL/plate ± activation (MRID No. 403896-08, Study No. 9583-0-401) (HCN).	Negative	Acceptable
Ames Test (Salmonella negative in strains TA1535, TA1537, TA1538, TA98 and TA100), Tested at up to 1000 μg/plate ± activation (MRID No. 00148033, Study No. R 5707). Unknown test material and purity.	Negative	Unacceptable
Structural Chromosomal Aberration In Vitro Cytogenetic CHO, Tested up to 283 μg/ml without activation, 1310 μg/ml with activation (MRID No. 403896-09, Study No. 9583-0-437). In this study, there was a potent positive response (twice the positive control levels), the response was dose-related (HCN).	**Positive (+/- activ.)**	Acceptable
Mouse Micronucleus assay, Tested up to 331 mg/kg, (MRID No. 403896-10, Study No. 10052-0-455) (HCN).	Negative	Acceptable
Rat Micronucleus assay, Tested up to 306 mg/kg (MRID No. 00148036, Study No. R 6012). (CaCN and another compound)	Negative	Unacceptable
Mouse Micronucleus assay, Tested up to 247 mg/kg (HCN)	Negative	Published study[6]
Other Genotoxic Effects Alkaline elution/rat hepatocyte assay (HCN)	Negative	Published study[7]
Unscheduled DNA Synthesis, Tested up to 143 μ/ml in F344 rat hepatocytes (MRID No. 403896-07, Study No. 9583-0-447) (HCN).	Negative	Acceptable
Sister Chromatid Exchange, Tested up to 330 mg/L (MRID No. 00148034, Study No. CL/78/120) (CaCN)	Negative	Unacceptable

[4]Haworth et al. 1983. Environ. Mutagen. 5 (S1): 3-142.

[5]Yoon et al. 1985. Environ. Mutagen. 7: 349-67.

[6]Menargues et al. 1984. Mutat. Res. 136: 127-9.

[7]Sina et al. 1983. Mutat. Res. 113: 357-91.

3. Subchronic and Chronic Toxicity

Reference: Accession No. 073726, HED Document No. 005681.

A 28-day range-finding feeding study in rats produced a NOEL of less than 100 ppm based on slight liver degeneration. Decreased body weight gain and hepatocellular injury were observed at 300 ppm and up. At ≥1000 ppm, decreased hemoglobin levels and increased relative liver weights were observed. At 3000 ppm, there was rough haircoat, decreased activity, decreased hemoglobin, increased relative liver and kidney weights and increased mortality. A 90-day feeding study in rats was conducted that resulted in NOEL/LELs of 20/60 ppm (approximately 1/3 mg/kg/day) based on histological changes in the thyroid gland.

Reference: MRID No. 415040-03, Study No. 2319-125.

A 92-week oral gavage study in rats resulted in NOEL/LELs of 2.5/7.5 mg/kg/day a.i. based on decreases in body weight gain, colloid in the thyroid, and decreased T3 and T4 levels. Numerous adverse effects were observed at 14 weeks including decreased RBC, hemoglobin, hematocrit, platelets, blood glucose and globulin levels, and increased lymphocyte count and GGT activity. Therefore, the initial dose levels of 2.5, 7.5 or 30 mg/kg/day were after decreased at 17 weeks. These effects were reversible by week 52. It was mentioned that there were no tumors in this study.

Reference: MRID Nos. 412888-02, 415665-01. Study No. 2319-121.

A one-year oral (gavage) study in dogs resulted in NOEL/LELs of 0.2/1.0 mg/kg/day based on decreased mean corpuscular volume and mean corpuscular hemoglobin, and an increase in pale areas of the spleen in males. In addition, at 5.0 mg/kg/day signs of toxicity included rough haircoat, desquamation of the skin, tremors, salivation, decreased body weight gain, decreased albumin, phosphorus, calcium and/or leucocyte counts as well as increased T4 levels and increased relative weight of the thyroid/parathyroid.

4. Structure-Activity Relationships

Reference: Memorandum from W. Greear to J. Miller/R. Ikeda dated November 8, 1990. Supplement to NCI Bioassays. HED DOC No. 8150.

Other than Calcium Cyanamide, which is essentially equivalent to Hydrogen Cyanamide, no other structurally-related analogs were identified.

The structures of Hydrogen Cyanamide and Calcium Cyanamide are shown below:

$H_2NC{\equiv}N$	$Ca{=}NC{\equiv}N$
Hydrogen Cyanamide	Calcium Cyanamide

5. Human Data

Calcium Cyanamide, a potent aldehyde dehydrogenase inhibitor, is used in alcohol aversion therapy to treat chronic alcoholism. Several reports have indicated that ground glass inclusion bodies of the hepatocytes have been observed in patients that were treated with Calcium Cyanamide. One patient treated for one year with 20 mg/day (≈0.286 mg/kg/day) showed the presence of inclusion bodies. There are reports that this effect is reversible. In rat studies the inclusion bodies are not observed, indicating that alcohol may be necessary in the production of the lesion.

F. Weight of the Evidence Considerations

The Committee considered the following observations regarding the toxicology of Hydrogen Cyanamide for a weight-of-the-evidence determination on its carcinogenic potential:

1. Hydrogen Cyanamide when administered to female CD-1 mice for 104 weeks in the drinking water was associated with a statistically significant increase in the incidence of total ovarian granulosa-theca cell tumors at 600 ppm (HDT) by pairwise comparison with controls ($p < 0.01$). There was also a positive trend ($p < 0.01$) for these tumors, and the incidence was well in excess of the upper end of the range of historical controls.

   There were no significant increases in any tumor types reported for male CD-1 mice administered Hydrogen Cyanamide up to 600 ppm in the drinking water for 100 weeks.

   Dosing was considered to be adequate in both sexes for assessing the carcinogenic potential of Hydrogen Cyanamide.

2. Calcium Cyanamide is rapidly and quantitatively converted to Hydrogen Cyanamide in solution and at the pH of human and rodent gut. Thus, Calcium Cyanamide is considered interchangeable with Hydrogen Cyanamide for the purposes of toxicity testing and evaluation of carcinogenicity potential.

3. In an NCI study, Calcium Cyanamide when administered in the diet to male B6C3F1 mice for 100 weeks was associated with an increase in malignant hemangiosarcoma at 2000 ppm (HDT). The incidence of hemangiosarcoma was twice that observed in historical control mice; however, the increase was not statistically significant by pairwise comparison with control mice although there was a statistically significant positive trend ($p < 0.01$).

   The increase in malignant lymphoma/leukemia in female B6C3F1 mice administered Calcium Cyanamide up to 2000 ppm in the diet for 100 weeks was not considered biologically significant by the CPRC.

   Dosing was considered to be adequate in both sexes for assessing the carcinogenic potential of Calcium Cyanamide.

4. In an NCI study, Calcium Cyanamide when administered in the diet at up to 200 ppm in male and up to 400 ppm in female F344 rats was not associated with significant increases in any tumors. Dosing was considered to be adequate in both sexes for assessing the carcinogenic potential of Calcium Cyanamide.

5. Hydrogen Cyanamide and Calcium Cyanamide have been tested in several mutagenicity assay systems. Hydrogen Cyanamide was strongly positive with and without activation in an in vitro cytogenetics study (twice that of the positive controls, increasing dose-response relationship). In addition, an Ames assay performed under the auspices of the NTP revealed weak positive results in Salmonella strain TA1535 in two laboratories. In a submitted study, negative results were obtained with the same strain (and others). Follow-up testing is necessary to ascertain if Hydrogen Cyanamide may present a risk to germ cells.

6. Other than Calcium Cyanamide, which is essentially equivalent to Hydrogen Cyanamide, no other structurally-related analogs were identified.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogenic Risk Assessment" (FR51: 33992-34003, 1986) for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that the classification for Hydrogen Cyanamide should be 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.

This 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 malignant hemangiosarcomas in male B6C3F1 mice, and activity in two mutagenicity assay systems.

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

SEP 2 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Subject: Hydrogen Cyanamide, Quantitative Risk Assessment, 2-Year Charles River CD-1 Mouse Dietary Study (in Drinking Water)

Caswell no.485A

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Summary

The unit risk, Q_1^* of hydrogen cyanamide, based upon female mice ovarian granulosa-theca tumors is 1.35×10^{-1} $(mg/kg/day)^{-1}$ in human equivalents. The dose levels in drinking water that occurred in this study were 0, 6.9, 16.9 and 49.0 mg/kg of hydrogen cyanamide. The effective proportions of ovary tumors observed in female mice were 3/59, 1/59, 7/57 and 13/56 for the above mentioned dose levels.

Background

In August, 1993 the Peer Review Committee recommended that the quantitative risk assessment for hydrogen cyanamide be estimated from female ovary granulosa-theca tumor rates.

The statistical evaluation (Hydrogen Cyanamide-Qualitative Risk Assessment Based on Charles River CD-1 Mouse Dietary Study, L.Brunsman 8/93) indicated significant increasing mortality in female mice with increasing doses in drinking water, of hydrogen cyanamide.

Female mice had significant dose related increasing trend in ovarian granulosa-theca tumor rates. There also was a significant increase in these tumors in the pair-wise comparisons of the highest (49.0 mg/kg) dose and the controls.

Dose-Response

Since female mouse mortality significantly increased with incremental doses of hydrogen cyanamide, the estimate of unit risk, Q_1^* was obtained by the application of Time-to-Tumor Multi-Stage model (Tox_Risk program, version 3.1 - K. Crump). In this version of K. Crump's program, the conversion to human risk by surface area adjustment uses a 70 kg. human instead of the 60 kg. as previously done, in order to estimate a more conservative risk.

The result of the estimate of unit risk, Q_1^* is as follows: Species,Mouse; Strain, Charles River CD-1; sex,female; tumor, ovarian granulosa-theca; Q1* $(mg/kg/day)^{-1}$ in human equivalents is 1.3×10^{-1}.

It is to be noted that Q_1^* $(mg/kg/day)^{-1}$ is an estimate of the upper bound (95%) on risk and that (as stated in the EPA Risk Assessment Guidelines) "the true value of the risk is unknown, and may be as low as zero".

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