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

SEP 24 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Hydrogen Cyanamide: PP #0F3868 - Request for an Exemption From the Requirement of a Tolerance and a Request for Registration of Dormex (Hydrogen Cyanamide) on Dormant Grape Vines (34555-E)

Tox. Chemical No.:	140
PC No.:	014002
DP Nos.:	D185537, D185564
Submission Nos.:	S430887, S430950

FROM: William B. Greear, M.P.H. William B. Greear 9/23/93
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Health Effects Division (H7509C)

TO: Joanne Miller/Eugene Wilson, PM Team #23
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THRU: Marion P. Copley, D.V.M., Section Head Marion P. Copley 9/23/93
Review Section IV, Toxicology Branch I
Health Effects Division (H7509C)
and
Karl P. Baetcke, Ph.D. Karl P. Baetcke 9/23/93
Chief, Toxicology Branch I
Health Effects Division (H7509C)

I. CONCLUSIONS:

The toxicity data base supports the registration of Dormex (Hydrogen Cyanamide) for a food or non-food use on dormant grape vines. The data base does not however, support an exemption from tolerance. The Carcinogenicity Peer Review Committee (CPRC) determined that Hydrogen Cyanamide is a group C carcinogen (see Section X. B.)

II. REQUESTED ACTION:

RD has requested that several new toxicological studies on Hydrogen Cyanamide be reviewed in order to establish an exemption from the requirement of a tolerance. The new studies submitted are:

1 of 121

81-5 Primary Dermal Irritation Study (MRID #415665-02, study #891330/STB 4/SE)

82-1 28-Day Range finding in Rats (MRID #422029-02, study #2319-123)

83-1 Chronic Toxicity in Rats (MRID #421784-04, study # HLA 2319-125)

83-4 Reproduction Study in Rats (MRID #415665-04, study #2319-126)

85-1 Metabolism (MRID #421784-07)

85-2 Dermal Absorption (MRID #415040-04, study #HLA 6265-100)

In addition several addenda were submitted to upgrade or clarify existing studies.

III. PRODUCT 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: Carbamide, 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 administration of Calcium Cyanamide are interchangeable with those that are dosed 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 if 140A[1]. The Chemical Abstract Registry Number (CAS No.) of Hydrogen Cyanamide is 420-04-2. The PC Code for Hydrogen Cyanamide is 014002, and the PC Code for Calcium Cyanamide is 014001.

The structure of Hydrogen Cyanamide is presented below:

$H_2NC{\equiv}N$

[1] There is some confusion in the old DERs and TOX one-liners as to the Caswell Number 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.

IV. REQUIREMENTS FOR TERRESTRIAL FOOD - USE (40 CFR 158.340)

Hydrogen Cyanamide #140
Updated: September, 1983

Technical:	Required	Satisfied
81-1 Acute Oral Toxicity	Y[2]	Y
81-2 Acute Dermal Toxicity	Y[2]	Y
81-3 Acute Inhalation Toxicity	Y[2]	Y
81-4 Primary Eye Irritation	Y[2]	Y
81-5 Primary Dermal Irritation	Y[2]	Y
81-6 Dermal Sensitization	Y[2]	Y
81-7 Acute Delayed Neurotox.(hen)	N	-
82-1 Subchronic Oral (rodent)	Y	Y[3]
82-1 Subchronic Oral (nonrodent)	Y	Y[4]
82-2 21-Day Dermal	N[5]	-
82-3 -Day Dermal	N	-
82-4 90 Day Inhalation	N	-
82-5 90-Day Neurotoxicity (hen)	N	-
82-6 90-Day Neurotoxicity (mammal)	N	-
83-1 Chronic Toxicity (rodent)	Y[6]	Y
83-1 Chronic Toxicity (nonrodent)	Y[6]	Y
83-2 Oncogenicity (2 species)	Y[6]	Y
83-3 Teratogenicity (2 species)	Y[7]	Y
83-4 Reproduction	Y	Y
83-5 Chronic/Oncogenicity	-	-
84-2 Mutagenicity - Gene Mutation	Y	Y
84-2 Mutagenicity - Struct. Chrom. Aber.	Y	Y
84-2 Mutagenicity - Other Genotoxic Effects	Y	Y
85-1 General Metabolism	Y[6]	Y
85-2 Dermal Penetration	N	Y
86-1 Domestic Animal Safety	N	-

Y - Yes; N - No.

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[2] Also required for end-use product. (Note: the technical is also the end-use product).

[3] The chronic (91-Week) oral gavage study in rats (MRID 421784-04, study # HLA 2319-125) may be used to satisfy the requirement for a subchronic rat study.

[4] The chronic study in the dog (#2319-121, 5/10/89) may be used to satisfy the requirement for a subchronic dog study.

[5] Not required since Hydrogen Cyanamide has a pH of 3.5 and is Toxicity Category I for dermal irritation.

[6] Not required for non-food use.

[7] Only one species is required for non-food use.

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Hydrogen Cyanamide #140
Updated: September 1993

V. TOXICOLCGY PROFILE

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

CASNELL#: 140
CAS-REG#: 156-62-7

P.C. CODE 014002- Cyanamide FILE LAST PRINTED: 09/23/93

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-1 Acute oral LD50 Species: rat CIVO-Cen. Inst Nutr & Food Res 2949 WN; 2/7/73	Cyanamide (Cyanamid L 500) purity approx. 50%	073726	LD50 = 0.285 (0.250 - 0.325) ml/kg. or approx. 300 mg/kg (based on 100% Cyanamid L500). Levels tested: 0.20, 0.25, 0.30, and 0.35 ml/kg (gavage) in Wistar derived strain.	2	Minimum 005681
81-2 Acute Dermal LD50 Species: rabbit Hazleton 23129-122; 2/9/88	Hydrogen Cyanamide 50%; Lot# 07/07/87	412888-01	Doses: 1, 2.5 & 4 mg/kg. Route: dermal in str NZW. LD50 (M) =1.7 ml/kg (850 mg/kg a.i.). 95% CL 1.1-2.7 ml/kg LD50 (F) = 1.4 ml/kg (700 mg/kg a.i.). 95% CL = 0.9-2.2 ml/kg.	2	Acceptable 008150
81-3 Acute inhalation LC50 Species: rat CIVO-Cen. Inst Nutr & Food Res R 4083; 5/73	Cyanamide-SKW Cyanamid L500 purity approx. 50%	073726	LC50 > 2.0 mg/L/4 hrs. Level tested: 2.0 mg/L (1 mg/L a.i.) str: Wistar derived.	3	Minimum 005681
81-4 Primary eye irritation Species: rabbit CIVO-Cen. Inst Nutr & Food Res R 4398; 6/74	Cyanamide-SKW Cyanamid L500 (50%)	073726	Slight corneal opacity. All displayed slight conjunctivitis on day 7. Str. NZW.	2	Minimum 005681
81-5 Primary dermal irritation Species: rabbit Central Inst Voedingsonderzoek 882-006-4; 1982	Cyanamide(SKW Cyanamid L500) 50% approx.purity		Corrosive at up to 7 days (end of test). Strain: NZW.	1	Minimum 005681
81-6 Dermal sensitization Species: guinea pig CIVO-Cen. Inst Nutr & Food Res V 82.096/220663; 3/82	Cyanamide-SKF Cyanamid F1000 (purity unknown)	073726	The test material is a strong sensitizer.		Guideline 005681

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P.C. CODE 014002- Cyanamide FILE LAST PRINTED: 09/23/93

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE/GRADE/ DOCUMENT#
83-2b Carcinogenic-2 year Species: mice Hazleton Labs, Europe 6001-556 3; 10/91	Hydrogen Cyanamide (50% a.i., w/w)	415665-02 421784-05	Dose levels: 70, 200 or 600 ppm in drinking water (Male: 5.0, 13.7 or 36.8 mg/kg/day a.i.; female: 6.6, 16.9 or 49.0 mg/kg/day a.i. NOEL = 70 ppm (M = 5 mg/kg a.i.; F = 6.9 mg/kg a.i.). LOEL = 200 ppm M = 13.7 mg/kg a.i.; F = 16.9 mg/kg a.i.) based on increased mortality in females & increased urinary bladder lesions and kidney lesions. In addition, at 600 ppm, there were increases in ovarian granulosa theca tumors, and ovarian stromal/luteal hyperplasia, decrease in body wt. (M).		Supplementary 008422 Minimum 010591
83-1(a) Gavage 20 month Species: rat Hazleton Lab America 2319-125; 04/15/91	Hydrogen Cyanamide 50% w/w a.i.; Lot# 07/07/87 and 12/04/88	421784-04	Aqueous hydrogen cyanamide was admin. via oral gavage to male & female Crl:CDBR rats at dose levels of 0, 2.5, 7.5 or 30 mg/kg/day active igred. hydrogen cyanamide for 16 weeks; dose levels were lowered to 0, 1, 2.5 or 7.5 mg/kg/day a.i. at week 17 because of excessive toxicity. Dosing continued through week 91. NOEL (systemic effects) = 2.5 mg/kg/day a.i. (both sexes). LOEL (systemic effects) = 7.5 mg/kg/day a.i. (the high dose) based on significant decreases in body weight gain and increases in the incidence of reduced colloid in the thyroid in males and females. There was also hunched posture, tremors and rough haircoat in both sexes. Treatment-related effects noted in one male included decreases in both thyroxine and triiodothyronine levels, and anemia (decreased red blood cell count, and hemoglobin and hematocrit levels). At the doses tested, there was no evidence of a carcinogenic effect.		Guideline ~~010592~~ 010591
83-1(b) Gavage-1 year Species: dog Hazleton 2319-121; 5/10/89	Hydrogen Cyanamide tech. (50% a.i. w/w)	412888-02	Administered by gavage to beagles at dose levels of: 0.2, 1.0 or 5.0 mg/kg/day a.i. NOEL = 0.2 mg/kg/day a.i. LOEL = 1.0 mg/kg/day a.i. (based on decreases in MCV and MCH in males and females, and an increase in pale areas of the spleen in males). In addition, at 5.0 mg/kg/day a.i. there were increases in males & female with rough haircoat & desquamation of the skin, tremors, salivation, decreases in body weight gain, leukocyte counts (M), albumin, calcium (F) phosphorous (M) & T4 (M) and an increase in the rel. weight of the thyroid-parathyroid (F), a possible decrease in sperm activity (M).		Guideline 008150 010591
83-3(a) Developmental Toxicity Study Species: rat Hazleton 2319-124; 5/2/89	Hydrogen Cyanamide 53% ai (w/w), 50% a.i. (w/w), Lot# 07/07/87	412888-06	Doses: 0, 5, 15 or 45 mg/kg/d ai; route oral (by gavage). Strain Crl:CDBP Maternal NOEL < 5 mg/kg a.i. Maternal LEL = 5 mg/kg (decr body wt gain) Developmental Tox NOEL = 15 mg/kg. Develop. Tox LEL = 45 mg/kg (based on increase in number of resorptions., decr. fetl body wt., incr in diaphramagtic hernia and wavy (bent) ribs.		Minimum 008150

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CASWELL#: 140
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P.C. CODE 014002- Cyanamide FILE LAST PRINTED: 09/23/93

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(b) Developmental Toxicity Study Species: rabbit CIVO-Cer Inst Nutr & Food Res B 84-017?; 5/89	Hydrogen Cyanamide 49% w/w a.i.; batch 250185	412888-05	Doses: 2, 6 or 18 mg/kg/day a.i. Route: oral (gavage) in NZW strain Maternal NOEL = 6 mg/kg. Maternal LOEL = 18 mg/kg a.i. based on decr. body wt gain. Developmental Tox. NOEL = 6 mg/kg. Develop. LEL = 18 mg/kg a.i. based on disintegration of liver structure and slight decr. in female size and weight.		Supplementary 000150 Minimum 010591
83-4 Reproduction-2 generation Species: rat Hazleton Lab America 2319-126; 04/19/9?	Hydrogen Cyanamide - 50% (w/w) a.i.; Lot# 07/07/87	415665-04	Crl:CD BR rats were dosed in the diet at levels of 0, 2.5, 7.5, or 30 mg/kg/day a.i. (Fo generation during premating) or 0, 1.25, 3.75 or 15 mg/kg/day a.i. during premating (F1 generation), gestation and lactation (Fo and F1 females). Parental NOEL = 1.25 mg/kg/day a.i. Parental LEL = 3.75 mg/kg/d a.i. based on significant decreases in body weight/weight gain and food consumption. Reprod. NOEL = not determined. Reprod. LOEL = 1.25 mg/kg/day a.i. based on decreased pup viability and body weight in both generations. In addition, at = 15 mg/kg/day a.i., decreased fertility was observed in both generations.		Minimum 01059.f
84-2(a) Mutagenic-Ames Species: salmonella Hazleton 9583-0-401; 10/21/87	Hydrogen Cyanamide 53% ai	403896-08	Doses: 0.10, 0.25, 0.5, 1, 5, 10 and 15 uL/plate in salmonella strains TA1535, TA1537, TA1538, TA98, and TA100. Results: negative.		Acceptable 006628
84-4 Mutagenic-unscheduled DNA synt Species: rat hepatocytes Hazleton 9583-447; 10/21/87	Hydrogen Cyanamide 53% ai	403896-07	Doses: 5.95, 11.9, 23.8, 47.6, 71.4, 95.2, 143 & 190 ug/ml in F344 rat hepatocytes. Results: Negative.		Acceptable 006628
84-2(b) Mut- Chrom. aberr. in vitro Species: CHO cells Hazleton 9583-0-437; 10/21/87	Hydrogen Cyanamide 53% ai		Doses: 42.4, 56.5, 141 and 424 ug/ml (w/o activation); 32.1, 42.8, 438, 875, 1310 and 1750 ug/ml with activ. Results: Negative.		Acceptable 006628
84-2(b) Mutagenic-micronucleus assay Species: mice Hazleton 10052-0-455; 10/21/87	Hydrogen Cyanamide (53% a.i.)	403896-10	Doses: 31.44, 157.4 and 330.5 mg/kg in ICR str. Results negative.		Acceptable 006628

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TOX ONELINERS

CASWELL#: 140
CAS-REG#: 156-62-7

P.C. CODE 014002- Cyanamide FILE LAST PRINTED: 09/23/93

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
Carcinogenicity- Peer Review Species: 09/15/93	Hydrogen Cyanamide		Peer Review Document - date: 09/15/93. Classification: Group C - possible human carcinogen. Q1* = 1.35 x 10exp-1 (mg/kg/day)exp-1.		010592
85-1 Metabolism Species: rat, dog rabbit Literature Rev.; Environ Corp. 09/05/91	14C-Hydrogen Cyanamide	421784-07	Hydrogen Cyanamide is rapidly absorbed, metabolized & excreted in the urine following oral, intravenous (i.v.), and 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 hrs, indicating that hydrogen cyanamide is rapidly metabolized and almost completely eliminated from the body. Negligible amounts of radioactivity were excreted as expired CO2. Following both oral and i.v. dosing in dogs, 62-83% of the dose was excreted in the urine within the first 24-27 hrs. 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.		Minimum 010591
85-2 Metabolism - dermal absorption Species: rat Hazleton Lab America HLA 6265-100; 12/08/89	Hydrogen Cyanamide 49%	415040-04	A single dermal dose of 0.1, 1, or 10 mg (8, 80, or 800 ug/cm2, respectively) 14C-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 14C-hydrogen cyanamide equivalents absorbed within 24 hrs were 1.79%, 2.84%, and 11.1% of the applied dose for the low, mid-, and high groups, respectively. The data can be utilized for risk assessment purposes as a worst case scenario.		Supplementary 010591

Calcium Cyanamide

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS

CASWELL#: 140A
CAS-REG#: 156-62-7

P.C. CODE 014001- Calcium cyanamide FILE LAST PRINTED: 09/23/93

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(b) Carcinogenic-2 year Species: mice National Cancer Inst. 79-1719; 1979	Calcium Cyanamide 48-66% Calcium cyanamide, purity 97%		Levels tested at 0, 500 or 2000 ppm (0, 3.5 or 150 mg/kg/day a.i.) in B6C3F1 str. Based on Cancer Peer Review (held 09/01/93) hemangiosarcomas in the HDT are of concern. The conversion data, calcium cyanamide ---> Hydrogen cyanamide, allows upgrading to minimum - 11/09/90 (1 Suppl. and a addendum in 9/93 memo).		Supplementary 005681 Minimum 008150 008162 010591
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat National Cancer Inst.	Calcium Cyanamide 48-66%; 97% pure	073727	Levels tested at: 0, 100 & 200 ppm (M); 0, 100 or 400 ppm (F). in F344 strain. No treatment related increase in tumors; letter (May 4, 1987) in reference to conference April 4, 1987. The agreemnet reached in conf-		Supplementary 005681 Min. (cancer)

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VI. DATA GAPS FOR TERRESTRIAL FOOD-USE:

No data gaps exist at this time.

VII. ACTION TAKEN TO REMOVE DATA GAPS AND OBTAIN ADDITIONAL INFORMATION:

No action is required at this time.

VIII. REFERENCE DOSE (RfD):

The RfD Committee recommended that a chronic Reference Dose (RfD) be established based upon a no-observable effect level (NOEL) of 0.2 mg/kg/day. Hematological and splenic changes were observed at 1.0 mg/kg/day in a long-term feeding study in dogs. An uncertainty factor (UF) of 100 was used to account for the inter-species extrapolation and intra-species variability. On this basis, the RfD was calculated to be 0.002 mg/kg/day (see memo of G. Ghali titled Hydrogen Cyanamide: RfD/Peer Review Report, dated September 15, 1993).

IX. PENDING REGULATORY ACTION:

None at present that TB-1 is aware of.

X. TOXICOLOGICAL ISSUES:

A. Dermal Irritation:

Three primary dermal irritation studies have been submitted. Study # V 84.090/230061 (dated 2/84) was considered to be "Invalid". Study No. B82-0061-4/1982 (dated 1982) was Core-Minimum Data with a Toxicity Category of I. The third study, No. 891330/D/STB 4/SE was Core-Guideline with a Toxicity Category of IV. An acute dermal study, No. R 4108 (dated 6/73), indicated that there was severe irritation (hemorrhage, edema, etc.). By the end of 1 to 2 weeks, rabbits in the 2.0 and 4.0 mg/kg displayed scaliness with necrotic areas of the treated skin areas. The above noted irritation is consistent with a pH of 3.5. Based on the above, the test material should be labeled as a Toxicity Category I for dermal irritation.

B. Carcinogenicity of Hydrogen Cyanamide:

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on September 1, 1993, to discuss and evaluate the weight-of-the-evidence for 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 be applied using the experimental animal tumor data for quantification of human risk (Q_1*). This decision was based on: the statistically significant increase of the incidence of ovarian granulosa-theca tumors in female CD-1 mice both by positive trend and pair-wise comparison with controls at the HDT; the positive trend in hemangiosarcomas in male B6C3F1 mice; and the positive genotoxicity results in two mutagenicity systems (see the Carcinogenicity Peer Review Committee Document on Hydrogen Cyanamide from E. Rinde dated September 15, 1993).

The Q_1* of Hydrogen Cyanamide based on ovarian granulosa-theca tumors in female mice is 1.35×10^{-1} (mg/kg/day)$^{-1}$ in human equivalents (see attachment 1, memorandum of B. Fisher dated September 2, 1993).

C. Developmental/Reproductive Toxicity - Risk Assessment:

It is recommended that for acute (ie. 1-3 days) exposure, the NOEL of 5 mg/kg/day a.i. from the rat developmental toxicity study (based on hypoactivity during the first 2 days of dosing at the LEL of 15 mg/kg/day a.i.) be used for establishing a margin of exposure (MOE). If the exposure is subchronic, it is recommend that the LEL for the reproductive toxicity study (1.25 mg/kg/day a.i.) be used with an additional factor of 3 (the estimated NOEL would be 0.41 mg/kg/day).

D. Mutagenicity:

There was mutagenic activity in two assay systems (see the Carcinogenicity Peer Review Document.)

E. Hydrogen Cyanamide - Necessity For a Tolerance:

At a meeting held on 8/23/93, it was decided 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 a fertilizer, **the proposed use of Hydrogen Cyanamide on dormant grape vines would be classified as a**

non-food use (see attachment 2, memorandum of G. Jeffrey Herndon, dated 8/26/93).

XI. STUDY REVIEWS FOR THIS ACTION

A. New Toxicological Studies:

1. Guideline Series 81-5: Primary Dermal Irritation (MRID# 415665-01); study # 891330D/STB 4/SE, December 22, 1989)

Conclusions: Slight erythema and/or edema was observed, but cleared by Day 3.
Toxicity Category: IV
Core Classification: Guideline
Study Acceptability: The study satisfies the requirement for a Guideline Series 81-5 Primary Dermal Irritation Study

2. Guideline Series 82-1: 28-Day Range Finding Study (MRID # 42[illegible]029-02, study #2319-123, 9/9/88)

Conclusions: Crl:CD rats (5/sex/dose) were treated orally (gavage) with aqueous Hydrogen Cyanamide for 28 days at doses of 0, 5, 10, 20 or 40 mg/kg/day a.i.
NOEL < 5 mg/kg/day a.i.
LOEL = 5 mg/kg/day a.i. [based on histological changes (decreased colloidal content and small closely packed follicular cells) in the male thyroid].
In addition, at 10 mg/kg/day a.i. there were increased incidences of bile duct hyperplasia and follicular cell hyperplasia in males, and an increase in pigmented splenic macrophages (females). At 20 mg/kg/day a.i., there was decreased colloidal content, increased follicular cell hyperplasia, and small, closely packed follicular cells in the thyroid (females); increased relative liver weight (males) and relative kidney weight (both sexes); decreases in body weight (males), body weight gain (both sexes), and total food consumption (males). At 40 mg/kg/day a.i., there were increases in splenic pigmented macrophages, renal tubular mineralization, relative thyroid/parathyroid weight, total bilirubin, BUN and decreases in RBCs, HGB, HCT, MCH and MCHC in males. In females, there were increases in relative liver weight and decreases in body weight, food consumption, HGB, HCT, MCH and MCHC in females.
Core Classification: Supplementary
Study Acceptable: The study is acceptable as a 28-day range-finding study.

3. Guideline Series 83-1a: Chronic Toxicity Study in Rats (MRID #421784-04, study #HLA 2319-125)

   Conclusions: In a chronic toxicity study, Crl:CD rats were administered aqueous Hydrogen Cyanamide (50 % w/w) daily by gavage at dose levels of 0, 2.5, 7.5 or 30 mg/kg/day a.i. for 16 weeks then 0, 1.0, 2.5 or 7.5 mg/kg/day a.i. for the remainder of the study.
   NOEL = 2.5 mg/kg/day a.i.
   LEL = 7.5 mg/kg/day a.i. based on decreases in body weight gain, reduced colloid in the thyroid and reduced T3 and T4 levels. Adverse effects at 14 weeks included: decreased RBC, hemoglobin and hematocrit.
   Carcinogenic potential: No treatment related increase in tumors.
   Core Classification: Guideline
   Study Acceptability: The study satisfies the requirement for a Guideline Series: 83-1a Chronic Toxicity Study in Rodents.

4. Guideline Series 83-4: Two-generation Reproduction Study in Rats (MRID# 415665-04, study # 2319-126, dated 4/17/90)

   Conclusions: In a two-generation reproduction study, Crl:CD rats were given aqueous Hydrogen Cyanamide (50% w/w) daily by gavage at dose levels of 0, 2.5, 7.5 or 30 mg/kg/day a.i. (F_0 generation during premating) or 0, 1.25, 3.75 or 15 mg/kg/day a.i. during premating (F_1 generation), gestation and lactation (F_0 and F_1 females.
   Parental NOEL = 1.25 mg/kg/day a.i.
   Parental LOEL = 3.75 mg/kg/day a.i., based on significant decreases in body weight/weight gain and food consumption.
   Reproductive NOEL = not determined
   Reproductive LOEL = 1.25 mg/kg/day a.i. based on decreased pup viability and decreased body weight in both generations.
   In addition, at 15 mg/kg/day a.i. and above, decreased fertility was observed in both generations.
   Core Classification: Minimum Data
   Study Acceptability: The study satisfies the requirement for a Guideline Series 83-4 Reproduction Study.

5. Guideline Series 85-1: Metabolism Study (Literature Review of Ten Studies) (MRID # 421784-07, 9/5/91).

Conclusions: 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 CO_2. Following both oral and i.v. 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 Hydrogen Cyanamide excreted in the urine of rats, rabbits, dogs and human was identified as N-acetylcyanamide. The conversion of Hydrogen Cyanamide to acetylcyanamide in vitro is catalyzed by an acetyl-S-CoA-dependent N-acetyltransferase present in rabbit and dog liver.
Core Classification: Minimum
Study Acceptability: Together these studies (data) satisfy the requirement for a Guideline Series 85-1 Metabolism Study.

6. Guideline Series 85-2: Dermal Absorption Study (MRID#415040-04, study #HLA 6265-100, 12/8/89)

Conclusions: A single 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 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. The data can be utilized for risk assessment purposes as a worst case scenario (approximately 10 %).
Core Classification: Supplementary
Study Acceptability: Study can be upgraded, but is not a required study. Therefore, there is no data gap for a Guideline Series 86-1 Dermal Absorption Study.

B. "Addenda" to previously reviewed studies: The sponsor submitted several addenda to further clarify or upgrade the results. The studies are:

1. Guideline Series 83-1b: Supplement 1 tc the "Chronic Toxicity Study in Dogs with Aqueous Hydrogen Cyanamide" Study No. 3319-121", (MRID #412888-02; TOX Doc. No. 008150).

   Conclusions: Aqueous Hydrogen Cyanamide (50% w/w, 53% w/v a.i.) was administered by gavage to groups of 4 male and 4 female dogs at dose levels of 0, 0.4, 2.0 or 10.0 mg/kg/day (equivalent to 0, 0.2, 1.0 or 5.0 mg/kg/day a.i.). [For the first two weeks the dogs were administered 0.2, 1.0 or 5.0 mg/kg/day of the 50% technical.]
   NOEL = 0.2 mg/kg/day a.i.
   LEL = 1.0 mg/kg/day a.i. (based on decreases in MCV and MCH in males and females, and an increase in pale areas of the spleen in males).
   In addition, at 5.0 mg/kg/day a.i. there were increases in males and females with rough haircoat and desquamation of the skin, tremors, salivation, decreases in body weight gain, leukocyte counts (males), albumin, calcium (females), phosphorous (males), T_4 (males), and an increase in the relative weight of the thyroid (females), a possible decrease in sperm activity (males).
   Classification: Core-Guideline
   Study Acceptability: the study satisfies the requirement for a Guideline Series 83-1b Chronic Toxicity in Dogs Study.

   [This supplement does not change the conclusions from the previous DER, it only clarifies them.]

2. Guideline Series 83-2b: Supplement 1 to MRID #415565-02, TOX DOC # 008422 (83-2b) titled "Hydrogen Cyanamide, Up to 104 Week Oral (Drinking Water) Carcinogenicity Study" (MRID # 421784-05, study # 6001-556/3, October, 1993)

   Conclusions: Hydrogen Cyanamide was administered in 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, 13.7 and 38.8 mg/kg/day a.i.; for females: 0, 6.6, 16.9 and 49.9 mg/kg/day a.i.).
   NOEL = 70 ppm (6.9 mg/kg/day a.i.)
   LEL = 200 ppm (16.9 mg/kg/day a.i.) 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 (38.8 mg/kg/day a.i.) in males there was decreased body weight gain (significant); in females 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 (49.9 mg/kg/day a.i.). Statistics were submitted by L. Brunsman (HED, memorandum dated August 16, 1993, in the Peer Review File).

3. Guideline Series 83-2b: Supplement 3 to TOX DOC # 008162, titled "Bioassay of Calcium Cyanamide for Possible Carcinogenicity" Study # (NIH) 79-1719, 1979)

   The Carcinogenicity Peer Review Committee (Document dated 9/15/93) concluded that hemangiosarcoma in the male mice were treatment related.

4. Guideline Series 83-2a and b: Addendum to the rat and mouse bioassay, titled "Stability of Calcium Cyanamide, Technical Grade, in Animal Diet with Regards to the NCI Bioassay "(MRID #415040-02, study #(NIH) 79-1719, TOX DOC #s 005681 and 008162), dated 1979.

   Conclusion: The stability of Calcium Cyanamide in the diet preparations used in the NCI studies are satisfactory based on recoveries at 7, 12 and 17 days.
   Core Classification: Minimum
   Study Acceptability: This study may be used to upgrade the classification of the NCI bioassays 83-2a and b to minimum. The studies however, were already upgraded to Minimum based on a report titled "Conversion Rate of Calcium (Technical Grade) to Hydrogen Cyanamide" dated 9/25/87 (refer to TOX DOC # 008150).

   NOTE: The dose levels noted in the original DER for the rat cancer study were incorrect for females. The male doses were 0, 100 and 200 ppm while the female doses were 0, 100 and 400 ppm.

5. Guideline Series 83-3b: Supplement 1 to TOX DOC # 008150, rabbit developmental study, titled "Preparation of Various Aqueous Cyanamide Solutions and Analysis of these Solutions for Actual Content, Homogeneity and Stability".

   Conclusions: NOEL (maternal toxicity) = 6 mg/kg a.i.
   LEL (maternal toxicity) = 18 mg/kg a.i. based on decreased body weight gain.

NOEL (developmental) = 6 mg/kg a.i.
LEL (developmental) = 18 mg/kg/a.i. based on disintegration of liver structure and slight decrease in fetal size and weight.
Core Classification: Minimum (data on the analytical concentration, stability and homogenicity data on the dosing solutions were provided in MRID #421784-06).
Study Acceptability: The study satisfies the requirement of a Guideline Series 83-3 Developmental Toxicity Study.

CIBA/TIBA

STUDY NO. 629 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN N.Z.W.-RABBITS

TABLE 11 TYPE OF VISCERAL ALTERATIONS IN FOETUSES AND THE NUMBER OF AFFECTED FOETUSES IN THE DIFFERENT GROUPS[1]

	mg cyanamide solution/kg b.w./day			
	0	4	12	36
Number of litters examined	22	20	21	18
Number of decapitated foetuses, completely examined	94	77	79	70
Number of intact foetuses examined, with exception of the head	87	67	70	60
Total number of foetuses examined	181	144	149	130
VISCERAL MALFORMATIONS				
Brain: hydrocephaly	0	1[2)]	0	0
Diaphragm: hernia	0	1[2)]	0	0
Liver: rupture of the left anterior lobe	0	1	0	0
Ovary: unilateral agenesis	0	0	1	0
Total number of foetuses with visceral malformations	0	2(2)	1	0
MINOR VISCERAL ANOMALIES				
Brain: small meningial haemorrhage/haemorrhage of the olfactory bulb	5(5)	7(5)	8(7)	10(6)
slightly dilated lateral ventricles: unilateral	0	1	0	0
bilateral	15(11)	7(5)	0	10(6)
slightly dilated 3rd ventricle	1	1	0	1

THIS IS A VERIFIED COPY OF AN ORIGINAL DOCUMENT. VERIFIED BY: DATE: 2/1-96

Table 11

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Attachment 1

010591

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

From: Bernice Fisher, Acting Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (H7509C)

Bernice Fisher 9/2/93

To: Marion P. Copley, D.V.M., Section Head
Review Section IV
Toxicology Branch I
Health Effects Division (H7509C)

Thru: William Burnam, Chief
Science Analysis Branch
Health Effects Division (H7509C)

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 $1.3 x 10^{-1}$.

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".

Attachment 2

M. Copley
FYI
010591

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

AUG 26 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: Hydrogen Cyanamide. Memo of Understanding Concerning the Classification of the Use of Hydrogen Cyanamide as a Plant Growth Regulator on Dormant Grape Vines as a Non-Food Use.

From: G. Jeffrey Herndon, Chemist
Tolerance Petition Section II
Chemistry Branch I - Tolerance Support
Health Effects Division (H7509C)

G. Jeffrey Herndon

Through: Debra Edwards, Ph.D., Chief
Chemistry Branch I - Tolerance Support
Health Effects Division (H7509C)

Debra Edwards

To: Joanne Miller/Eugene Wilson, PM Team 23
Fungicide-Herbicide Branch
Registration Division (H7505C)

and

Albin Kocialski, Head
Registration Section
Chemical Coordination Branch
Health Effects Division (H7509C)

Background

In PP#0F3868, the petitioner, Siemer and Associates Inc., on behalf of the registrant, SKW Trostberg AG, is proposing that a Section 3 registration and an exemption from the requirements of a tolerance be established for the residues of the plant growth regulator hydrogen cyanamide (H_2NCN), in or on grapes and grape by-products.

No tolerances currently exist for the plant growth regulator hydrogen cyanamide. Hydrogen cyanamide, under the trade name Dormex®, is used to promote uniform bud break in grapes and various stonefruit 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. In moist soil, hydrogen cyanamide itself readily hydrolyzes into urea, ammonium, and nitrate. For this reason, both have been used as sources of nitrogen fertilizer dating back to the early 1900's. An estimated total in excess of 300,000 tons of calcium cyanamide is produced annually worldwide, with approximately 80-85% of this amount used for fertilizer. Based on the estimated use of 200-400 lbs. of nitrogen per acre on grapes grown in CA and AZ (the proposed use area in this petition), this would equate to 300-600 lbs. of hydrogen cyanamide (or 575-1150 lbs. of calcium cyanamide) per acre.

Previously, calcium cyanamide was registered for various uses including a herbicide on various crops at rates up to 4,260 lbs.ai./A. (90 days prior to seeding); a fungicide on beans, celery, crucifers, lettuce, potatoes, and tomatoes (30 days prior to planting) at rates up to 440 lbs.ai./A.; and as a defoliant for cotton (7 days before harvest) at rates up to 28.5 lbs.ai/A. These were all classified as non-food uses when they were originally granted, and the last of such registrations expired on 1/22/91 (product 54555-1, SKW Trostberg AG.) due to non-payment of fees.

For the proposed use as a plant growth regulator on dormant grapes, the maximum label rate is 17.2 lbs.ai./A. The proposed typical preharvest interval would be 130 days. There is an inherent disincentive for use of hydrogen cyanamide as a plant growth regulator on dormant grape vines other than the one proposed: use of rates greater than the proposed rate, or application too close to normal bud break can result in phytotoxicity or burned buds.

Plant Metabolism

A metabolism study on grapes has been previously submitted and reviewed by the Agency. Detached grape leaves were labeled via the petiole by immersing in 10-25μL containg 2-3μg of ^{14}C-hydrogen cyanamide. After uptake, the leaves were kept in a distilled water reservoir and allowed to metabolize in the light in a controlled laboratory environment for a 24 hour period. During the metabolic period, gases were collected in gas dispersion bottles containing H_3PO_4 (for hydrogen cyanamide) or KOH (for CO_2).

The laboratory extracted the leaves with dilute oxalic acid and separated the soluble portion into various classes using ion exchange resins. The lab analyzed the fractions using multiple chromatography systems [TLC (visualized using radiation detector (RTLC), X-ray film, UV, and various reagents (ninhydrin, potassium permanganate, aniline hydrogen phthalate, etc.)), paper, and high resolution ion exchange chromatography (HRIEC)], derivatization or isotope dilution and recrystallization to a specific radioactivity,

and quantitation using combustion and liquid scintillation counting (LSC).

After 24 hours of metabolism, no hydrogen cyanamide was detected in the leaf samples. Based on the efficacious use of hydrogen cyanamide as a fertilizer, this should not be surprising. The petitioner provided data to indicate that the single carbon of HC is rapidly incorporated into the general carbon pool of the grape plant, as shown in Table 1.

Table 1

^{14}C Metabolites Found in Grape Leaves After Feeding ^{14}C-Labeled Hydrogen Cyanamide

fraction	metabolites found
volatile	$^{14}CO_2$ (accounted for about 12% of the applied dose). No ^{14}C-hydrogen cyanamide was found.
neutral	At least 9 different components were present. Glucose, fructose, and sucrose were identified and accounted for about 70% of the total radioactivity in the neutral fraction.
insoluble	Cellulose was identified and determined to account for 29% of the radioactivity contained in the insoluble fraction.
anion	Malic acid (about 26% of the radioactivity in the anion fraction) and citric acid (about 15%) were identified.
cation	A single unidentified peak accounted for about 70% of the radioactivity contained in the cation fraction that did not correspond to any of the common amino acids, guanylurea, guanylthiourea, guanidine, nor dicyanodiamide. Tests show that the compound did not contain a carbonyl or acidic moiety. Separate studies show that this may be a transient intermediate. A study using young grape apices tentatively identified guanyl-type cations shortly after HC uptake, but these metabolites failed to persist and were absent after a 3 day metabolic period (label was increasingly incorporated into a protein fraction). In cotton plants at 8 hours, an unknown cation was found that was not present at longer intervals (at longer intervals, the label was found in amino acids, protein, and insoluble products)

The results indicate that no residues of parent compound should be present at harvest (130 day PHI). It is likely that most of the applied hydrogen cyanamide is incorporated into natural plant components by the pathways shown in Attachment I.

Livestock Metabolism

No animal metabolism studies were submitted in support of this petition. The registrant has requested a waiver from the requirement of lactating goat and laying hen metabolism studies based on no significant exposure of livestock or poultry to hydrogen cyanamide. This is based on the non-detectable residue levels of hydrogen cyanamide in grapes as a result of proposed use of Dormex® on dormant grape vines.

Meeting of 8/23/93

On 8/23/93, the following members of HED met to discuss the various possibilities (e.g. setting a limit of quantitation tolerance, tolerance exemption, or non-food use) for classification of the use of hydrogen cyanamide on dormant grape vines:

Karl Baetcke	-	TOX1
Paul Chin	-	TOX1
Marion Copley	-	TOX1
Debbie Edwards	-	CBTS
Nan Gray	-	CCB
Jeffrey Herndon	-	CBTS
Rick Loranger	-	CBTS
Andy Rathman	-	CCB
Dick Schmitt	-	Deputy Division Director

During the course of the meeting, we 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.

Based on the use of hydrogen cyanamide on dormant grape vines being classified as a non-food use, it was decided that a meeting with the HED Metabolism Committee would not be necessary.

Attachment I: Proposed Metabolism of Hydrogen Cyanamide in the Grape Leaf

cc: PP#0F3868, RF, SF, Metabolism Committee file, circu., G.J. Herndon, E. Haeberer (section head), Nan Gray (CCB/HED), M. Copley (TOX1/HED), members of the HED Metabolism Committee:
[K. Baetcke (TOX1/HED), A. Protzel (TOX2/HED), R. Engler (HED), R. Schmitt (HED), G. Ghali (SAB/HED), M. Metzger (CBRS), R. Loranger (CBTS).

RDI: Section Head: E. Haeberer: 8/25/93,
Branch Senior Scientist: R.A. Loranger: 8/25/93.

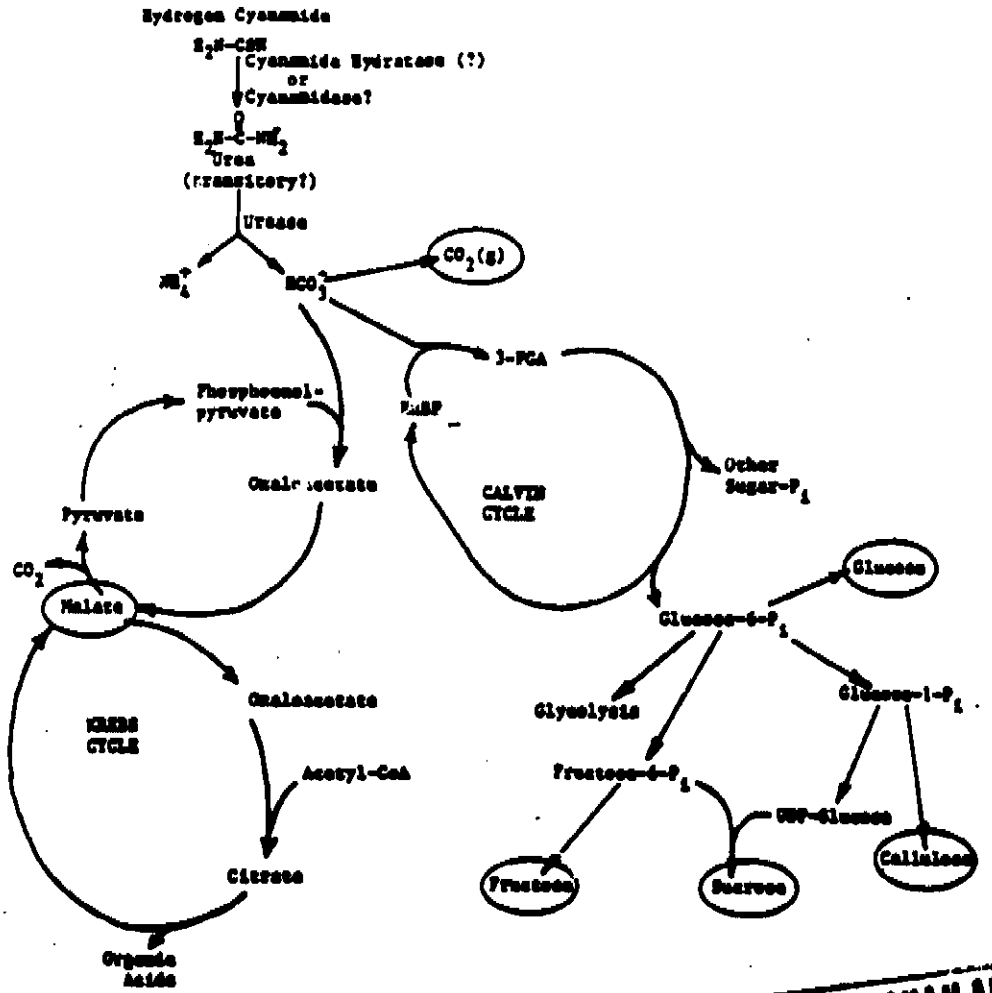
H7509C: CBTS: G.J. Herndon: 305-6362: CM#2, Rm. 804C: 8/24/93.

ATTACHMENT I

Figure 11

Metabolism of Hydrogen Cyanamide in the Grape Leaf

(Taken from MRID# 412888-09)



010591

Reviewed by: William B. Greear, M.P.H. William B. Greear 1/6/93
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M.
Review Section IV, Toxicology Branch I (H7509C) Marion Copley 1/6/93

DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 81-5
Primary Dermal Irritation

Tox. Chem. No.: 140 ~~366D~~
PC No.: 014002
MRID No.: 415665-01

TEST MATERIAL: Aqueous Hydrogen Cyanamide 49% w/w, clear cclorless material
Batch No. 110889 (=520 g/l)

SYNONYMS: Carbimide, Carbodiimide, Amidocyanogen, Cyanogenamide, SKW 83010, CAS 420-04-2

STUDY NUMBER: 891330D/STB 4/SE

SPONSOR: SKW Trostberg AG
D-8223 Trostberg, FRG
(Siemer & Associates, Inc.)

TESTING FACILITY: Huntingdon Research Center Ltd.
Huntingdon, Cambridgeshire, England

TITLE OF REPORT: Irritant Effects on Rabbit Skin of Aqueous Hydrogen Cyanamide 49% w/w

AUTHOR: Michael P. Liggett

REPORT ISSUED: December 22, 1989

CONCLUSIONS: Slight erythema and/or edema clearing by Day 3.

TOXICITY CATEGORY: IV

Classification: Core-Guideline

Study Acceptability: The study satisfies the requirement for a Guideline Series 81-5 Primary Dermal Irritation Study.

MATERIALS AND METHODS:

[Procedures based on OECD Guideline for Testing of Chemicals No. 404 "Acute Dermal Irritation/Corrosion". Adopted May 12, 1981.]

Six male New Zealand White rabbits weighing 2.2 to 2.9 Kg were obtained from Froxfield Farms (U.K.) Ltd., Petersfield, Hampshire, England and were acclimated to laboratory

conditions. The animals were individually housed in metal cages with perforated floors in one animal room maintained at approximately 19°C, relative humidity of 30 to 70%, a 12-hour on/12-hour off light cycle with 19 air changes per hour. Food (SDS Standard Rabbit Diet) and water were available ad libitum. Approximately 24 hours prior to dosing, the hair was removed from the dorso-lumbar region of each rabbit exposing approximately a 10 cm^2 area. One-half ml of the test material was applied under a 2.5 cm^2 gauze pad and placed on one intact site on each animal. Each site was wrapped with an elastic adhesive dressing for a 4-hour period. The animals were not restrained. At the end of the 4-hour period the dressings were removed from the treatment sites and the backs washed with water to remove any residual test material. The sites were evaluated 30 minutes after removal of the patches and on Days 2, 3 and 4. The grading system employed was essentially that of Draize, with ratings from 0 to 4 for erythema and eschar formation and from 0 to 4 for edema.

RESULTS:

Very slight (5 rabbits) to well-defined erythema (1 rabbit) and very slight edema (5 rabbits) were observed 30 minutes after removal of the patches. Two rabbits exhibited very slight erythema on Day 2. No irritation was subsequently observed.

FINAL

DATA EVALUATION REPORT

AQUEOUS HYDROGEN CYANAMIDE

Study Type:
28-Day Oral Toxicity Study in Rats

Prepared for:

Office of Pesticide Programs
Health Effects Division
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

April 15, 1993

Principal Reviewer: Patricia A. Bittner — Date 5/13/93
Patricia Bittner, M.S.

Independent Reviewer: C. Rabe — Date 5/13/93
Carrie Rabe, Ph.D.

QA/QC Manager: Sharon A. Segal — Date 5/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-78
Clement Number: 203
Project Officer: Caroline Gordon

EPA Reviewer: William Greear, M.P.H.
Review Section 4, Toxicology Branch I,
Health Effects Division

Signature: William B Greear
Date: 5/20/93

EPA Section Head: Marion Copley, D.V.M.
Review Section 4, Toxicology Branch I,
Health Effects Division

Signature: Marion Copley
Date: 6/4/93

010591

DATA EVALUATION REPORT

STUDY TYPE: 28-Day oral toxicity study in rats (82-1)

TEST MATERIAL: Aqueous hydrogen cyanamide

PC Code: 014002

Tox. Chem. Number: ~~2665~~ 140

MRID Number: 422029-02

SYNONYMS: Cyanamide, Dormex, Carbamide, Carbodiimide, Amidocyanogen, Cyanogenamide, SKW 83010

STUDY NUMBER: 2319-123

SPONSOR: SKW Trostberg, AG, Trostberg, West Germany

TESTING FACILITY: Hazleton Laboratories America, Inc., Rockville, MD

TITLE OF REPORT: 28-Day Repeated Dose Oral Toxicity Study with Aqueous Hydrogen Cyanamide in Rats

AUTHOR: M. Osheroff

STUDY COMPLETION DATE: September 9, 1988

CONCLUSIONS: Crl:CD® rats (5/sex/dose) were treated orally (gavage) with aqueous hydrogen cyanamide for 28 days at doses of 0, 5, 10, 20, and 40 mg hydrogen cyanamide/kg/day.[a.i.] NOEL < 5 mg hydrogen cyanamide/kg/day.[a.i.] LOEL = 5 mg hydrogen cyanamide/kg/day, based on histological changes (decreased colloidal content and small, closely packed follicular cells) in the male thyroid.

In addition, at 10 mg hydrogen cyanamide/kg/day[a.i.], there were increased incidences of bile duct hyperplasia (males), pigmented splenic macrophages (females), and follicular cell hyperplasia (males).

At 20 mg hydrogen cyanamide/kg/day[a.i.], there were increased incidences of decreased colloidal content, follicular cell hyperplasia, and small, closely packed follicular cells in the thyroid (females); significant increases in relative liver weight (males) and relative kidney weight (both sexes);

significant decreases in mean body weight (males), mean body weight gain (both sexes), and total food consumption (males).

At 40 mg hydrogen cyanamide a.i./kg/day, there were increased incidences of splenic pigmented macrophages (males) and renal tubule mineralization (males); significant increases in relative liver weight (females) and relative thyroid/parathyroid weight (males); significant increases in total bilirubin (males) and blood urea nitrogen (males); significant decreases in body weight (females) and food consumption (females); significant decreases in erythrocyte count (males), hemoglobin (both sexes), hematocrit (both sexes) mean cell hemoglobin (males) and mean cell hemoglobin concentration (males).

CORE CLASSIFICATION: Supplementary. This study was intended as a range-finding study and was not intended to fulfill Guideline requirements.

A. MATERIALS AND METHODS

1. Test Article Description

Name: Aqueous hydrogen cyanamide
Lot number: 04/22/87
Purity: 50% w/w (53% w/v a.i. hydrogen cyanamide)
Date received: May 5, 1987
Physical property: Yellow liquid
Stability: Not reported
Storage conditions: 5°C

2. Dose Preparation

Weighed amounts of the test material were mixed with Polar® distilled water to achieve desired concentrations; the solution was administered daily to rats via oral gavage in a dose volume of 10 mL/kg. Test solutions were prepared weekly; no data were provided on the storage conditions. Control animals received the same amount of vehicle (distilled water) as treated animals.

Purity and stability of the test material were not evaluated in this study, but data on these parameters are on file with the sponsor. The concentration and stability of the test material in the dosing solutions were verified using ultraviolet (UV) spectrophometry of the reaction product of hydrogen cyanamide with ammonium disodium-pentocyanoamminferrate and sodium carbonate. Samples from each weekly preparation of dosing solutions were assayed for concentration. In addition, 8-day stability analyses were performed on control and high-dose solutions.

Results: Concentrations of the test material in the dose solutions ranged from 97.33 to 105.4% of target. Stability analyses showed no loss of test material over 8 days.

3. Animals

Species: Rat
Strain: Crl:CD®Br
Age: 39 Days at start of exposure
Weight at initiation: Males--162-205 g on day 1 of exposure
Females--144-172 g on day 1 of exposure
Source: Charles River Laboratories, Raleigh, NC

Group assignment: Animals were acclimated for 10 days and were assigned by sex to the following groups using a computer-generated randomization procedure based on body weight:

Test Group	Dose Active Ingredient (mg/kg/day)	Number of Animals	
		Males	Females
1 Control	0	5	5
2 Low dose	5	5	5
3 Mid-1 dose	10	5	5
4 Mid-2 dose	20	5	5
5 High dose	40	5	5

Doses of aqueous hydrogen cyanamide administered to treated animals were 10, 20, 40, and 80 mg/kg/day, but are presented as doses of active ingredient (hydrogen cyanamide).

Each animal was housed individually and identified by a tail tattoo. Purina Certified Rodent Chow® #5002 and tap water were available ad libitum. Both diet and water were analyzed (by supplier and unknown source, respectively), and the study author reported that there were no contaminants present that would be expected to interfere with the study results. Environmentally controlled animal rooms were maintained at temperatures of 72±6°F, relative humidity of 50±20%, and a 12-hour photoperiod.

Rationale for dose selection: Not reported, however, this study will be used as a range-finding study for subsequent chronic and developmental toxicity studies in rats.

4. Statistics

Body weights (initial and terminal), body weight changes, food consumption, clinical pathology (except cell morphology), and organ weight data were analyzed statistically. Levene's test of homogeneity of variances was used, and if variances were heterogenous, a series of transformational analyses (log^{10}, square, square root, reciprocal, and angular) were used. If homogeneity was not achieved, rank-transformed data were analyzed. ANOVA was performed on all rank-transformed heterogenous data, and all homogenous data; if significant, Dunnett's test was performed.

5. Quality Assurance

A signed Statement of Compliance with OECD GLPs, dated September 9, 1988 and November 27, 1991, was provided.

A signed Quality Assurance Statement with a list of quality assurance inspections, dated September 9, 1988, was provided.

B. METHODS AND RESULTS

1. General Observations

Animals were observed twice daily for mortality, moribundity, and signs of toxicity. Physical examinations with detailed clinical observations were performed weekly.

Results: No treatment-related mortalities were observed for either sex at any dosage level. One female (20 mg/kg/day) was found dead on study day 29, but this was not considered to be treatment related. Rough haircoat was found in 3/5 males and 1/5 females at 40 mg/kg/day, but was not noted in any other group.

2. Body Weights/Food Consumption

Body weight and food consumption data were recorded weekly throughout the study.

Results: Mean body weight, body weight gain and food consumption data for weeks 0-4 are presented in Tables 1 and 2. Mean body weights were significantly ($p<0.05$) lower at 20 mg/kg/day (15%, males) and at 40 mg/kg/day (26%, males; 20%, females). Mean body weight gains (weeks 0-4) were significantly ($p<0.05$) decreased at 20 mg/kg/day (30%, males; 36%, females) and at 40 mg/kg/day (53%, males, 57%, females). Total food consumption (weeks 1-4) was significantly ($p<0.05$) decreased in males at 20 mg/kg/day (13%) and at 40 mg/kg/day (25%).

3. Ophthalmoscopic Examination

Ophthalmologic examinations were not conducted.

4. Clinical Pathology

Animals were fasted and blood was collected from the orbital sinus under ketamine anesthesia (hematology) and from the abdominal aorta under sodium pentobarbital anesthesia (clinical chemistry) prior to necropsy. The checked (X) parameters were determined:

(a) Hematology

X Hematocrit (HCT)
X Hemoglobin (HGB)
X Leukocyte count (WBC)
X Erythrocyte count (RBC)
X Platelet count
Reticulocyte count (RETIC)
X Cell morphology
X Corrected leukocyte count (COR WBC)
X Leukocyte differential count
X Mean corpuscular HGB (MCH)
X Mean corpuscular HGB concentration (MCHC)
X Mean corpuscular volume (MCV)
Coagulation: thromboplastin time (PT)

Results: A summary of selected hematologic parameters is presented in Table 3. Significant decreases in the following hematologic parameters were observed at 40 mg/kg/day[a]: erythrocyte count (males), hemoglobin (both sexes), hematocrit (both sexes), mean cell hemoglobin (males), and mean cell hemoglobin concentration (males). A significant increase in monocyte count was noted in males at 40 mg/kg/day[a]; however, the biological significance of this is unknown.

(b) Blood (clinical) chemistry

Electrolytes

X Calcium
X Chloride
Magnesium
X Phosphorus
X Potassium
X Sodium

Enzymes

Alkaline phosphatase (ALP)
Cholinesterase
Creatine phosphokinase
Lactic acid dehydrogenase
X Serum alanine aminotransferase (SGPT)
X Serum aspartate aminotransferase (SGOT)
X Gamma glutamyltransferase (GGT)

Other

X Albumin
X Albumin/globulin ratio
X Blood creatinine
X Blood urea nitrogen
Cholesterol (total)
X Globulins
X Glucose
X Total bilirubin
Direct bilirubin
X Total protein
Triglycerides

Results: A summary of selected clinical chemistry parameters is presented in Table 4. Significant increases in mean total bilirubin (0.2 mg/dL, compared to 0 mg/dL in controls) and blood urea nitrogen (73%) were noted in males at 40 mg/kg/day[a]. Significant decreases in globulin levels (16%) were observed at 20 and 40 mg/kg/day[a] in males; the magnitude of the decrease was similar at both doses. All other changes were considered incidental.

(c) Thyroid function tests:

Thyroid stimulating hormone (TSH)
Thyroxine (T_4)
Triiodothyronine (T_3)

Results: No compound-related effects were observed for either sex at any dosage level. The increases (≈100%) observed in thyroid stimulating hormone values at 40 mg/kg/day[a] in both sexes were nonsignificant due to the high variability among the animals.

5. Sacrifice and Pathology

All animals were euthanized by exsanguination under sodium pentobarbital anesthesia and necropsied after 28 days of treatment. The checked (X) tissues were collected and preserved in 10% neutral buffered formalin. Histological examinations of these tissues were performed for all control and high-dose animals (0 and 40 mg/kg/day,[a] respectively). Examination of the spleen, liver and thyroid was also performed for the 5-, 10-, and 20- mg/kg/day groups, and for gross lesions of animals from all groups. In addition, double-checked (XX) organs were weighed.

Digestive System		Cardiovascular/Hematologic	Neurologic
Tongue		Aorta	XX Brain (w/brainstem)
Salivary glands	X	Heart	Peripheral nerve
Esophagus		Bone marrow	(sciatic nerve)
X Stomach		Lymph nodes	Spinal cord
Duodenum	X	Spleen	(three levels)
Jejunum		Thymus	Pituitary
Ileum			Eyes
Cecum		Urogenital	(optic nerve)
Colon			
Rectum	XX	Kidneys	Glandular
XX Liver		Urinary bladder	
Gallbladder	XX	Testes	XX Adrenals[a]
Pancreas	XX	Epididymes	Lacrimal gland
		Prostate	Mammary gland
Respiratory		Seminal vesicle	XX Thyroids[a]
		Ovaries	XX Parathyroids[a]
Trachea		Uterus	Harderian glands
Lungs			

Other

Bone (sternum and femur)
Skeletal muscle
Skin
X All gross lesions and masses

[a]Weighed post fixation

Results:

(a) Organ weights

Compound-related effects were observed in liver and thyroid; possible effects were observed in kidney. Selected relative organ weight data are presented in Table 5. Significant increases were noted in relative liver weight at 20 (22% males) and 40 mg/kg/day[a] (62%, males; 19%, females), relative thyroid/parathyroid weight at 40 mg/kg/day[a] (49% males), and relative kidney weight at 20 (11%, males and females) and 40 mg/kg/day[a] (28%, males; 23%, females). Adrenal and thyroid weights were determined post-fixation; the effect of this process on these organ weights was not determined. Significant increases in relative brain weight at 40 mg/kg/day[a] (25% males) were noted; however, since there is a lack of histopathological evidence of toxic effect for the brain, these relative organ weight changes are believed to be a result of decreased body weight. Significant decreases in absolute testes/epididymes weight was noted at 20 (14%) and 40 mg/kg/day (15%) (data not shown). This change was not noted in relative testes/epididymes weight and is not dose related, thus, it is believed to be incidental.

(b) Macroscopic pathology

No compound-related gross findings were observed for either sex at any dosage level.

(c) Microscopic pathology

Dose-related increases in the incidence and severity of thyroid effects and in the incidence of liver and spleen effects were observed. A summary of histopathological findings in selected organs is presented in Table 6. Histopathology of the female (20 mg/kg/day[a]) found dead on day 29 revealed suppurative pyelonephritis and chronic hepatic and urinary bladder inflammation, but this was not considered compound related.

In the thyroid, decreased colloidal content was observed in males beginning at 5 mg/kg/day[a], but was increased in severity only at 40 mg/kg/day[a]; it was observed in females beginning at 20 mg/kg/day. Follicular cell hyperplasia was observed beginning at 10 mg/kg/day in males, and at 20 mg/kg/day[a] in females. In addition, small and closely packed follicles were observed beginning at 5 mg/kg/day in males and 20 mg/kg/day[a] in females.

An increased incidence of bile duct hyperplasia was observed in males beginning at 10 mg/kg/day[a]. An increased incidence of pigmented macrophages was observed the spleens of males at 40 mg/kg/day[a] and females beginning at 10 mg/kg/day[a]. Renal tubule mineralization was observed in males at 40 mg/kg/day[a], but not in males at 0 mg/kg/day.

Table 1. Selected Body Weights and Body Weight Gains (g ± S.D.) in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a,b,c]

Dose (mg hydrogen cyanamide/kg)	Mean Body Weight at Week: 0		1		4		Mean Body Weight Gain at Weeks: 0–4	
Males								
0	179	14	234	19	369	29	190	19
5	191	9	249	12	389	35	198	33
10	184	13	242	18	356	31	173	22
20	181	15	227	18	314*	33	133*	20
40	186	4	226	8	275*	20	89*	21
Females								
0	156	8	184	13	244	24	88	17
5	161	10	185	10	242	18	81	15
10	163	5	185	6	233	1	70	5
20	158	9	172	12	214	14	57*	7
40	159	12	171	16	197*	15	38*	5

[a]Data were extracted from Study No. 2319-123, Table 2.

[b]Mean S.D.

[c]N = 5 Animals/sex/dose

*Significantly different from controls ($p<0.05$)

Table 2. Mean Food Consumption (g ± S.D.) in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a,b]

010591

Dose (mg hydrogen cyanamide/kg)	Mean Food Consumption at Week: 1		2		3		4		Total Food Consumption at Week: 1-4	
					Males					
0 (N)[c]	177.2 (5)	12.1	189.7 (5)	15.1	196.0 (4)	11.8	188.1 (5)	11.7	760.5 (4)	50.0
5 (N)	186.6 (4)	7.6	196.8 (4)	5.2	198.1 (4)	6.9	186.7 (4)	14.5	766.3 (4)	28.2
10 (N)	191.3 (5)	12.2	193.3 (5)	14.3	192.1 (5)	13.9	177.7 (4)	10.9	768.2 (4)	36.3
20 (N)	170.1 (5)	6.8	179.1 (5)	13.4	164.7 (4)	13.2	149.7 (5)	11.7	659.8* (4)	38.7
40 (N)	164.4 (5)	18.2	148.6 (5)	13.6	135.2 (5)	9.7	124.0 (5)	10.2	572.2* (5)	41.9
					Females					
0 (N)	144.7 (5)	16.4	141.4 (5)	21.1	146.1 (5)	26.1	137.5 (4)	24.3	552.5 (4)	79.5
5 (N)	140.1 (3)	10.1	138.7 (4)	10.0	141.0 (4)	9.9	139.5 (3)	15.1	585.8 (2)	20.3
10 (N)	137.4 (5)	5.9	137.9 (5)	5.3	139.6 (4)	10.3	128.5 (4)	8.0	536.4 (3)	4.4
20 (N)	125.3 (5)	7.4	121.3 (5)	10.9	122.8 (5)	11.9	113.3 (5)	16.3	482.6 (5)	42.3
40 (N)	132.6 (4)	13.0	119.2 (3)	11.4	113.4 (2)	8.3	110.0 (5)	8.2	466.5 (2)	50.0

[a]Data were extracted from Study No. Z319-123, Table 3.

[b]Mean S.D.

[c]N = Number of animals

*Significantly different from controls ($p<0.05$)

**Significantly different from controls ($p<0.01$)

Table 3. Selected Hematology Results in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a,b]

010591

Parameter:	Dose Level (mg hydrogen cyanamide/kg)[a.i.] 0		5		10		20		40	
No. of animals evaluated										
Males	5		5		5		5		4[c]	
Females	5		5		5		4		5	
Males										
RBC (MI/UL)	8.2	0.3	7.8	0.3	8.1	0.3	8.4	0.3	7.5*	0.5
(%)[d]									(9)	
Hemoglobin (g/dL)	17.2	0.3	16.6	0.5	16.5	0.3	17.3	0.4	14.5*	0.7
(%)[d]									(16)	
Hematocrit (%)	47.4	1.2	46.0	1.0	46.2	0.8	47.6	1.4	41.0*	1.8
(%)[d]									(14)	
Mean Corpuscular Hemoglobin (pg)	20.9	0.5	21.4	0.8	20.5	0.7	20.7	0.6	19.3*	0.6
(%)[d]									(8)	
Mean Corpuscular Hemoglobin Concentration (g/dL)	36.2	0.4	36.2	0.5	35.8	0.4	36.3	0.4	35.3*	0.2
(%)[d]									(3)	
Monocyte count (TH/UL)(%)	0.1	0.10	0.0	0.05	0.0	0.09	0.2	0.19	0.4*	0.20
Females										
RBC (MI/UL)	7.9	0.2	8.1	0.3	8.4	0.3	8.3	0.1	7.5	0.4
Hemoglobin (g/dL)	16.4	0.2	16.9	0.9	17.3	0.6	16.9	0.5	15.1*	0.6
(%)[d]									(8)	
Hematocrit (%)	45.7	0.6	46.6	1.9	47.2	1.7	46.5	1.3	42.5*	0.9
(%)[d]									(7)	
Mean Corpuscular Hemoglobin (pg)	20.6	0.5	21.0	0.5	20.7	0.3	20.3	0.7	20.3	0.5
Mean Corpuscular Hemoglobin Concentration (g/dL)	35.9	0.2	36.3	0.7	36.7	0.4	36.4	0.5	35.6	0.7
Monocyte count (TH/UL) (%)	0.1	0.04	0.1	0.11	0.2	0.20	0.2	0.18	0.3	0.11

[a]Data were extracted from Study No. 2319-123, Table 4.

[b]Mean S.D.

[c]The blood sample from one male at this dose was clotted.

[d]Percentage diffence from control values

*Significantly different from control ($p<0.05$)

Table 4. Selected Clinical Chemistry Results in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a,b]

010591

Parameter:	Dose Level (as hydrogen cyanamide/kg) 0		5		10		20		40	
No. of animals evaluated										
Males	5		5		5		5		5	
Females	5		5		5		4		5	
Males										
Total bilirubin (mg/dL)	0.0	0.0	0.0	0.09	0.0	0.05	0.0	0.05	0.2*	0.13
Blood urea nitrogen (mg/dL)	11.0	1.0	11.0	2.2	11.0	1.6	13.0	1.1	19.0*	5.2
Globulin (g/dL)	1.9	0.2	1.9	0.1	1.8	0.1	1.6*	0.1	1.6*	0.2
Females										
Total bilirubin (mg/dL)	0.1	0.09	0.1	0.08	0.1	0.11	0.1	0.10	0.2	0.11
Blood urea nitrogen (mg/dL)	11.0	2.1	16.0	6.4	15.0	4.3	15.0	3.3	18.0	7.2
Globulin (g/dL)	1.8	0.1	1.7	0.2	1.7	0.3	1.7	0.2	1.6	0.2

[a]Data were extracted from Study No. 2319-123, Table 5.

[b]Mean S.D.

*Significantly different from control ($p<0.05$)

Table 5. Selected Relative Organ Weights in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a]

010591

	Dose Level (mg hydrogen cyanamide/kg)				
	0	5	10	20	40
No. of animals evaluated					
Males	5	5	5	5	5
Females	5	5	5	4	5
Organ:					
			Males		
Brain	0.60 0.05	0.58 0.05	0.61 0.07	0.68 0.05	0.75* 0.04
Liver	2.93 0.18	3.14 0.23	3.21 0.31	3.56* 0.24	4.74* 0.51
Kidney	0.82 0.06	0.81 0.03	0.83 0.05	0.91* 0.06	1.04* 0.06
Thyroid/ Parathyroid	0.0065 0.0015	0.0057 0.0008	0.0079 0.0017	0.0076 0.0009	0.0097* 0.0015
			Females		
Brain	0.84 0.12	0.86 0.04	0.89 0.05	0.91 0.03	0.98 0.08
Liver	3.01 0.16	2.88 0.31	2.96 0.17	3.08 0.17	3.57* 0.33
Kidney	0.80 0.05	0.82 0.03	0.83 0.06	0.88* 0.03	0.98* 0.05
Thyroid/Parathyroid	0.0088 0.0027	0.0070 0.0018	0.0073 0.0018	0.0081 0.0023	0.0111 0.0018

[a]Data were extracted from Study No. 2319-123, Table 8.

*Statistically different from control ($p<0.05$)

Table 6. Selected Histopathological Results in Rats Dosed Orally with Aqueous Hydrogen Cyanamide for 28 Days[a]

010591

Organ/Observation:	Dose Level (mg hydrogen cyanamide/kg a.i) 0	5	10	20	40
No. of animals evaluated[b]					
Males	5	5	5	5	5
Females	5	5	5	5	5
Males					
Thyroid					
Decreased colloid content					
Slight	0	0	0	0	1
Minimal	0	1	5	5	0
Moderate	0	0	0	0	1
Moderately severe	0	0	0	0	3
Follicular cell hyperplasia					
Slight	0	0	0	0	3
Minimal	0	0	5	5	0
Moderate	0	0	0	0	2
Small and closely packed follicles	0	2	5	5	5
Spleen					
Pigmented macrophages	0	0	0	0	5
Liver					
Bile duct hyperplasia	0	0	1	2	5
Kidney					
No. of animals examined	5	1	1	0	5
Tubule mineralization	0	0	0	0	3
Females					
Thyroid					
Decreased colloid content					
Slight	0	0	0	0	2
Minimal	0	0	0	1	1
Moderate	0	0	0	0	1
Follicular cell hyperplasia					
Slight	0	0	0	0	3
Minimal	0	0	0	1	1
Small and closely packed follicles	0	0	0	2	4
Spleen					
Pigmented macrophages	0	0	2	4	5
Liver					
Bile duct hyperplasia	0	0	0	0	0
Kidney					
No. of animals examined	5	1	0	1	5
Tubule mineralization	2	1	0	0	0

[a]Data were extracted from Study No. 2319-123, Table 9 and Appendix 7.

[b]Examination of thyroid, spleen and liver

C. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS

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The data reporting was thorough, and the summary means that were validated were supported by individual animal data. The reviewers agree with the study author that the decreases in body weight, body weight gains, and food consumption were dose related, and that the rough coat observed in the high-dose animals was reflective of their compromised condition. Red cell mass appeared to be decreased in both sexes at high dose as indicated by significant decreases in erythrocyte hematology parameters (red blood cells, hemoglobin, hematocrit). The author suggests that this may be the result of erythrocytic hemolysis, which would also support the slight increase in total serum bilirubin among some of these animals. The increase in blood urea nitrogen (BUN) and tubule mineralization suggest some renal effect in males. However, whether the mineralization in males is a compound-related effect is equivocal since it was observed sporadically among some of the control and low-dose females that were examined.

Increases in relative organ weights were correlated with histopatho-logical effects in the liver, thyroid, and possibly kidney. The thyroid, liver, and spleen have been identified as target organs of toxicity. Histopathological examination of the thyroid showed follicular cell hyperplasia, decreased colloid content, and small/closely packed follicles. Although there were no statistically significant changes in thyroid function parameters, thyroid stimulating hormone values were doubled in both males and females at the high dose compared to controls. These changes were not significant, however, due to the high variability among these animals. The reviewers believe that the bile duct hyperplasia, which was observed in males, may represent toxic insult to the liver. There is validity to the study author's suggestion that the pigmentation in splenic macrophages may be due to phagocytized erythrocytes.

Deficiencies in the study include a lack of information on the stability of the test material and on the intermediate level dosing solutions. Lack of stability is frequently encountered at lower dose levels, and cannot be ruled out without this information.

Based on changes in the thyroid (males), the NOEL could not be determined; the LOEL was 5 mg hydrogen cyanamide/kg/day.[sic]

FINAL

010591

DATA EVALUATION REPORT

HYDROGEN CYANAMIDE

Study Title: Chronic Toxicity Study in Rats with Aqueous Hydrogen Cyanamide

Chronic Oral Toxicity Study in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

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April 15, 1993

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Work Assignment Number: 2-78
Clement Number: 204
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DATA EVALUATION REPORT

STUDY TYPE: Guideline Series 83-1: Chronic Oral Toxicity in Rodents - Rat

MRID NUMBER: 421784-04

TOX CHEMICAL NO: 140

PC CODE: 014002

TEST MATERIAL: Hydrogen Cyanamide

SYNONYM: None listed

STUDY NUMBER: HLA Study No. 2319-125

SPONSOR: SKW Trostberg, AG; Trostberg, West Germany

TESTING FACILITY: Hazleton Laboratories America, Inc., Rockville, MD

TITLE OF REPORT: Chronic Toxicity Study in Rats with Aqueous Hydrogen Cyanamide

AUTHOR: Osheroff, M.R.

REPORT ISSUED: April 15, 1991

CONCLUSIONS: Aqueous hydrogen cyanamide was administered via oral gavage to male and female Crl:CDBR rats at dose levels of 0, 2.5, 7.5, or 30 mg/kg/day active ingredient hydrogen cyanamide for 16 weeks; dose levels were lowered to 0, 1, 2.5 or 7.5 mg/kg/day a.i. at week 17 because of excessive toxicity. Dosing continued through week 91.

NOEL (systemic effects) = 2.5 mg/kg/day a.i. for both sexes.

LOEL (systemic effects) = 7.5 mg/kg/day a.i. (the high dose) based on significant decreases in body weight gain and increases in the incidence of reduced colloid in the thyroid in males and females.

Other treatment-related effects noted in males administered 7.5 mg/kg/day a.i. included decreases in both thyroxine and triiodothyronine levels, and anemia (decreased red blood cell count, and hemoglobin and hematocrit levels).

Clinical observations in both sexes receiving 7.5 mg/kg/day a.i. consisted of hunched posture, tremors and rough haircoat.

At the doses tested there was no evidence of a carcinogenic effect.

CORE CLASSIFICATION: Core Guideline. This study satisfies the Guideline requirements for a chronic study in rodents (83-1).

A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Aqueous hydrogen cyanamide (50% w/w, 53% w/v active ingredient)

Lot numbers: 07-07-87 (received July 27, 1987); 12-04-88 (received April 26, 1988)

Purity: 50% w/w Active ingredient (Sponsor analysis). Dosing formulations were not adjusted for purity.

Physical property: Clear, colorless liquid

Stability: Stable for at least 76 weeks (refrigerated or stored at room temperature

Storage condition: Under refrigeration

2. Test Material Preparation and Dosing Regimen

Aqueous hydrogen cyanamide was administered once daily for 7 days/week by oral gavage at a dosing volume of 10 mL/kg. Dosing volumes were adjusted based on individual animal's most recent body weight. The test material was prepared in purified water (control article) weekly and stored under refrigeration until use. To prepare dosing solutions, the appropriate amount of technical material was weighed into a beaker and then transferred to a volumetric flask. Beakers were rinsed with distilled water, and the rinse was added to the volumetric flasks. The contents of the flasks were diluted with the appropriate amount of control article and the flasks were inverted to obtain a solution.

3. Test Material Analyses for Purity and Stability

The purity of the test material in the dosing solutions was not verified in the study.

The stability of the test material in the low- and high-dose solutions (refrigerated samples) was determined at days 0, 7, and 10. Further stability analyses at week 76 were repeated on the low-dose solutions that were sampled at days 0 and 7 because the dose levels were lowered for all groups at week 17; both refrigerated and room temperature samples were analyzed for stability. All stability analyses were performed in duplicate. Results showed that the test

material in the low- and high-dose solutions were stable when stored either refrigerated or at room temperature for at least 76 weeks; test solutions were within ±10% of target levels.

The concentration of test material in the dosing solutions was determined weekly for weeks 1-4 and biweekly through week 92. Results of concentration analyses indicated that test solutions were within ±10% of target levels with the exceptions of the high-dose solution (88.58% of target) at week 8 and the mid-dose solution (117.3% of target) at week 38.

4. Animals

Species: Rat
Strain: Crl:CDBR
Age and weight at study initiation: 44 Days; males -- 212.9-243.8 g; Females -- 154.2-191.4 g.
Source: Charles River Laboratories, Raleigh, NC
Housing: Individual
Environmental conditions: Temperature: 72±6°F
Humidity: 50±20 %
Air changes: Not reported
Photoperiod: 12-hour light/dark cycle

Animals were acclimated to laboratory conditions for 2 weeks and were randomly assigned (on the basis of body weight) to the following groups:

Group	No. of Animals Male	No. of Animals Females	Weeks 0-16 Dose Levels (mg/kg/day) Technical	Weeks 0-16 Dose Levels (mg/kg/day) Active Ingredient	Weeks 17-91 Dose Levels (mg/kg/day)[a,b] Technical	Weeks 17-91 Dose Levels (mg/kg/day)[a,b] Active Ingredient
1 Control	20	20	0	0	0	0
2 Low	20	20	5	2.5	2	1
3 Mid	20	20	15	7.5	5	2.5
4 High	20	20	60	30.0	15	7.5

[a]Due to severe weight loss in the mid- and high-dose animals and general debilitation in health status, dose levels were decreased for all dose groups. Dose levels were decreased at week 17.
These doses are used in the rest of this DER
[b]The study was terminated at week 92 because of low survival in the mid-dose females.

Dose selection: A rationale for dose selection was not provided.

5. Statistics

Survival data through week 91 were examined by life table analysis (National Cancer Institute Package). Trend analysis of survival was evaluated at the 5.0% one-tailed probability level. Clinical

hematology (except cell morphology), clinical chemistry, body weight, organ weight, and food consumption data were evaluated using Levene's test for equality of variances. Log transformations were performed when necessary. If variances were homogeneous, analysis of variance (ANOVA) was used to analyze the data. If homogeneity of variance was not established, analyses were performed on rank-transformed data. Significant differences between treated and control animals were analyzed by Dunnett's test. Statistically significant differences were assumed at $p \leq 0.5$.

6. Quality Assurance

A statement of compliance with Good Laboratory Practice Standards (signed and dated 5/8/91) and a Quality Assurance Statement (signed and dated 4/15/91) were provided in the study report.

B. METHODS AND RESULTS

1. General Observations: Animals were observed twice daily for mortality and moribundity. Cage-site observations for clinical signs of toxicity were performed daily. In addition, detailed physical examinations were conducted on each animal at every weighing interval.

Results:

The number of scheduled deaths (SD) and unscheduled deaths (UD) occurring during the first 92 weeks of the study were reported (text table 2, page 29 of the study report) for each dose group as follows:

Group (mg/kg/day) a.i.	Male SD	Male UD (% Survival)[b]	Female SD	Female UD (% Survival)[b]
0	9	10[a] (47)	15	5 (75)
1	12	8 (60)	13	7 (65)
2.5	14	6 (70)	8	10[a] (44)
7.5	14	5[a] (74)	14	2[a] (88)

[a]Number excludes animals that were killed accidentally.
[b] Denominator doesn't include accidental deaths

Although the study author indicated that cumulative survival data through week 91 were evaluated statistically, the results of statistical analyses were not presented. After 91 weeks on the study, the survival (adjusted for accidental deaths) of the males was 47% in the controls, compared with 60%, 70%, and 74% for the low-, mid-, and high-dose groups, respectively. Among the females, the survival after 91 weeks on the study was 75% in the controls, compared with 65%, 44%, and 88% for the low-, mid-, and high-dose groups, respectively. The study author indicated that statistical analysis of survival data revealed a significant decrease (compared

to control) in the mid-dose females (the statistical data were not presented). The cause of death varied among dose groups and included accidents, neoplasms, and inflammatory processes, none of which were attributable to treatment.

Hunched posture, tremors, and rough haircoat were the most notable treatment-related clinical observations in most of the high-dose rats, particularly during weeks 9-17. The incidences of these signs were markedly decreased after the dose levels were lowered at week 17. Other clinical signs were incidental.

2. Body Weight/Food and Water Consumption/Test Material Intake:

Individual body weight data were recorded at initiation of treatment, once a week during the first 24 weeks of study and every four weeks thereafter. Food consumption (g/week) was recorded once a week during the first 16 weeks of the study and every four weeks thereafter. Food efficiency was calculated at weekly intervals for the first 16 weeks of treatment. Water intake was not monitored.

Results:

Body weight: Tables 1 and 2 summarize data on mean body weights and mean body weight gains at selected intervals. Statistically significant decreases in mean body weights were seen in the mid-dose males at weeks 4 (6.9%), 13 (14.8%), and 16 (15.8%) when compared to those of controls. Statistically significant decreases in mean body weights were seen in the high-dose males at weeks 4 (24.9%), 13 (41.6%), 16 (44.3%), 52 (30%), and 91 (32.1%) when compared to those of controls. Statistically significant decreases in mean body weights were seen in the mid-dose females at weeks 4 (5.8%) and 16 (6.5%) when compared to those of controls. Statistically significant decreases in mean body weights were seen in the high-dose females at weeks 4 (14.8%), 13 (23.7%), 16 (25.7%), 52 (11.3%), and 91 (16.5%) when compared to those of controls.

Mean body weight gains were decreased dose-dependently in males and females when compared to controls, particularly during the first 16 weeks of treatment. In males, statistically significant decreases were observed in the mid-dose males at weeks 0-16 (24%) and in the high-dose males at weeks 0-13 (66%) and weeks 0-16 (69%). In females, statistically significant reductions were observed in the mid-dose females at weeks 0-16 (14%) and in the high-dose females at weeks 0-13 (58%) and at weeks 0-16 (59%).

As a result of the marked decreases in body weight gains, dose levels were reduced at week 17. Following the decrease in dose levels, mean body weight gains in the mid- and high-dose males and females were higher than those of controls at weeks 16-91. The increase was statistically significant ($p \leq 0.05$) in the high-dose females (58% increase compared to control). At weeks 52-91, a marked decrease in body weight gain was observed in the high-dose males (-7.4 g compared to a gain of 41.5 g in the controls).

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TABLE 1. Mean Body Weights (g) at Selected Intervals for Rats Dosed With Aqueous Hydrogen Cyanamide for 91 weeks[a]

Weeks	Dose Level (mg/kg)[b] 0	2.5	7.5	30.0
		Males		
0	231.1	226.7	226.7	226.2
13	598.0	575.6	509.5*	349.2*
24	690.7	667.6	612.6	478.4
52	770.2	767.0	707.4	539.6*
91	787.4	760.1	747.6	534.4*
		Females		
0	176.2	174.7	175.0	177.9
13	303.1	300.0	290.2	231.2*
24	331.9	326.0	333.1	293.0
52	378.3	379.1	376.3	335.7*
91	468.1	466.1	456.7	391.0*

[a]Data were extracted from Table 4A of the study report.
[b]Dose levels were lowered to 0, 1, 2.5, or 7.5 mg/kg/day at week 17

*Significantly different from control value, $p \leq 0.05$.

TABLE 2. Mean Body Weight Gains (g) at Selected Intervals for Rats Dosed With Aqueous Hydrogen Cyanamide for 91 weeks[a]

Weeks	Dose Level (mg/kg)[b] 0	2.5	7.5	30.0
		Males		
0-13	366.9	349.0	282.9*	123.0*
0-52	538.0	540.3	480.7	312.3*
16-52	149.4	167.3	184.5	193.4*
16-91	196.3	184.9	225.8	192.7
52-91	41.5	31.1	48.4	-7.4
		Females		
0-13	126.9	125.3	115.3	53.6*
0-52	202.0	204.4	201.6	158.9*
16-52	63.7	76.4	84.6	101.2*
16-91	154.8	166.1	160.9	155.4
52-91	90.9	86.9	85.1	55.5

[a]Data were extracted from Table 4B of the study report.
[b]Dose levels were lowered to 0, 1, 2.5, or 7.5 mg/kg/day at week 17

*Significantly different from control value, $p \leq 0.05$.

TABLE 3. Mean Total Food Consumption (g) at Selected Intervals for Rats Dosed With Aqueous Hydrogen Cyanamide for 91 weeks[a]

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	Dose Level (mg/kg)[b] a.i.			
Weeks	0	2.5	7.5	30.0
		Males		
1-13	2326.3	2307.0	2219.0	1718.0*
1-52	4331.3	4442.5	4247.2	3532.1*
16-52	1719.1	1800.8	1753.2	1582.0*
16-88	3410.7	3449.2	3378.3	3065.6*
52-88	1846.8	1866.6	1840.6	1661.5*
		Females		
1-13	1547.4	1615.1	1508.3	1357.6*
1-52	3047.5	3118.5	2930.3	2791.7
16-52	1285.5	1258.6	1248.5	1215.4
16-88	2606.3	2479.3	2415.7	2369.0*
52-88	1462.0	1358.4	1370.7	1336.7

[a]Data were extracted from Table 5B of the study report.
[b]Dose levels were lowered to 0, 1, 2.5, or 7.5 mg/kg/day a.i. at week 17

*Significantly different from control value, $p<0.05$.

Food consumption: Mean food consumption (g/week) was slightly decreased in the high-dose males and females during the first 16 weeks of the study; the decreases were not statistically significant. However, total food consumption (g) was significantly ($p \leq 0.05$) decreased in the high-dose males at weeks 1-13, 1-16, 1-52, 16-52, 16-88, and 52-88; food intakes ranged from 73 to 92% of control values during these intervals. Total food consumption was significantly ($p \leq 0.05$) decreased in the high-dose females at weeks 1-13, 1-16, and 16-88; food intakes were 88 to 91% of the control values at these weekly intervals. Table 3 summarizes selected data on total food consumption. There was no apparent effect on food consumption in the low- and mid-dose groups. Mean feed efficiency in the mid- and high-dose males and females were lower than control during weeks 0-16; thereafter, an improvement in feed efficiency was seen.

3. Ophthalmologic examinations: Ophthalmoscopic examinations were conducted on all animals at pretest and at week 92.

There were no treatment-related ocular effects.

4. Clinical Pathology:

Hematological analyses were performed on the first ten surviving animals per sex, per group at weeks 14, 27, 52, 79, and at termination. Clinical chemistry analyses were conducted in these same animals at weeks 14, 27, 52, and at termination. A second group of animals (10/sex/group) was evaluated for thyroid function (triiodothyronine, thyroxine, and thyroid stimulating hormone). Leukocyte differential blood count and cell morphology were examined in control and high-dose animals at the same intervals as the hematological analyses, and in the low- and mid-dose animals at weeks 14 and 92. Blood was collected from the orbital sinus. Animals were fasted overnight prior to blood collections. The checked (x) parameters were examined.

(a) Hematology

X	Hematocrit (HCT)*	X	Leukocyte differential count
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*		Mean corpuscular HGB concentration (MCHC)
X	Erythrocyte count (RBC)*		Mean corpuscular volume (MCV)
X	Platelet count*		Coagulation: thromboplastin time (PT)
	Reticulocyte count (RETIC)		
X	Red cell morphology		

* = Recommended by Subdivision F (November 1984) Guidelines

Results:

Table 4 summarizes selected hematology data. Hemoglobin concentrations, hematocrit values, and red blood cell counts were significantly decreased (9.6-13.4%) in the high-dose males at week 14; however, no significant changes in these parameters were

TABLE 4. Representative Hematological Parameters in Rats Dosed With Aqueous Hydrogen Cyanamide for 91 Weeks[a]

Parameter/ Weekly Interval	Dose Groups (mg/kg/day)[b] 0	2.5	7.5	30.0	0	2.5	7.5	30.0
	Males				Females			
RBC (mi/UL)								
Week 14	8.41±0.43	8.49±0.29	8.64±0.37	7.43±0.61*	8.00±0.52	8.38±0.40	8.39±0.43	7.56±0.39
27	8.73±0.38	8.66±0.22	8.64±0.33	8.37±0.36	8.08±0.25	8.46±0.35	8.25±0.61	8.12±0.25
52	8.42±0.62	8.45±0.35	8.44±0.26	8.38±0.38	7.76±0.33	7.75±0.75	7.64±0.60	7.59±0.52
92	7.58±0.86	7.71±0.84	7.89±0.53	7.34±0.96	7.37±0.70	7.56±0.71	7.50±0.67	7.21±1.08
Hemoglobin (g/dL)								
Week 14	15.7±1.12	16.0±0.70	16.4±0.67	13.6±1.3*	15.8±1.23	16.7±0.68	16.4 ±0.88	15.2±0.83
27	15.9±0.71	15.8±0.54	15.8±0.63	15.3±0.47	15.9±0.61	16.7±0.54	15.9 ±1.13	15.6±0.58
52	15.5±0.77	15.4±0.65	15.5±0.66	15.1±0.63	15.3±0.72	15.3±1.33	14.8 ±1.21	14.9±0.61
92	13.8±18.0	14.3±1.56	15.1±0.97	13.1±2.09	14.8±1.22	15.1±1.46	14.9 ±1.16	14.3±1.82
Hematocrit (%)								
Week 14	44.7±2.61	45.0±1.97	46.8±2.05	40.4±3.56*	44.5±3.15	46.5±1.92	46.1 ±2.31	44.4±2.22
27	46.5±1.89	45.5±1.44	46.6±2.01	44.8±1.16	46.0±1.50	47.6±1.07	46.3 ±2.89	45.7±1.93
52	44.9±2.49	44.6±2.03	45.4±1.69	44.1±1.41	44.2±2.15	43.7±3.59	43.1 ±3.40	43.8±1.83
92	40.3±4.67	41.7±4.10	43.9±2.55	38.5±5.51	43.1±3.49	43.7±4.15	43.4 ±3.35	42.1±4.92

(continued)

TABLE 4 (Continued)

Parameter/ Weekly Interval	Dose Groups (mg/kg/day)[a,b]							
	0	2.5	7.5	30.0	0	2.5	7.5	30.0
	Males				Females			
Platelet (TH/UL)								
Week 14	1093± 77.4	1032±167.9	915± 54.1*	838±117.8*	1104±110.0	909±108.0*	906±100.4*	653±153.6*
27	1116±122.9	1091±130.7	965± 85.6*	988± 46.9*	1042±106.6	1011±158.0	950±126.5	840±163.3*
52	1155±179.7	1048±167.6	966±144.1	926±257.8	1069±102.3	908±212.3	918±203.9	892±134.2
92	1160±305.4	932±295.2	934±186.2	955±257.0	882±371.6	815±208.2	855±138.2	659±275.7
Lymphocyte count (TH/UL)								
Week 14	10.6±2.58	13.0±1.45	15.0±2.12*	16.1±4.03*	8.8±2.54	10.8 ±2.47	11.1 ±2.33	12.9 ±3.77*
27	10.5±1.84	------[c]	------	11.7±2.11	7.8±1.76	------	------	10.0 ±2.62
52	9.9±2.24	------	------	12.4±2.82	6.8±1.22	------	------	8.6 ±3.42
92	9.5±2.15	9.5±3.12	11.5±4.23	12.5±7.64	5.6±1.79	8.1 ±2.14*	7.4 ±1.36	9.7 ±5.00*

[a]Data were extracted from Table 7 of the study report.
[b]Dose levels were lowered to 0, 1.0, 2.5, or 7.5 mg/kg/day at week 17.
[c]Data not recorded.

*Significantly different from control values, $p<0.05$.

noted at any of the remaining intervals, possibly reflecting the lowering of dose levels at week 17. Platelet counts were significantly decreased in the mid- and high-dose males at weeks 14 (16.3% and 23.3%, respectively) and 27 (13.5% and 11.5%, respectively), in all female dose groups at weeks 14 (18-41%), and in the high-dose females at week 27 (19.4%); however, platelet counts were still within the normal range. No significant differences in platelet counts were noted after 27 weeks.

In the mid- and high-dose males, leukocyte, corrected leukocytes, and absolute lymphocyte count were significantly increased (38-52%) only at week 14. In the high-dose females, absolute lymphocyte count was significantly increased (47%) only at week 14. All of the above-mentioned hematological changes were treatment related. These changes were resolved by week 52.

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
X Chloride*	Albumin/globulin ratio
Magnesium	X Blood creatinine*
X Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	X Total bilirubin*
	Direct bilirubin
X Alkaline phosphatase (ALP)	X Total protein*
Cholinesterase (plasma, RBC, brain)	Triglycerides
X Creatine phosphokinase	
Lactic acid dehydrogenase	
X Serum alanine aminotransferase (SGPT)*	
X Serum aspartate aminotransferase (SGOT)*	
X Gamma glutamyltransferase (GGT)	

* = Recommended by Subdivision F (November 1984) Guidelines

Results:

Table 5 summarizes selected clinical chemistry data. Glucose levels were significantly ($p<0.05$) decreased in all treated females (20-40%) and in the mid- and high-dose males (28% and 32%, respectively) at week 14; the significant decreases persisted in the high-dose males (week 27) and in the high-dose females (weeks 27 and 52). Among other clinical chemistry parameters measured at week 14, significant differences compared with control included decreased albumin (9.4% and 13% in mid- and high-dose females, respectively), decreased total protein (7.1% and 14.3% in the mid- and high-dose females), decreased globulin in high-dose males (21%) and females (22%), increased albumin/globulin ratio in high-dose males (50%), decreased

TABLE 5. Selected Clinical Chemistry Findings in Rats Dosed With Aqueous Hydrogen Cyanamide for 91 Weeks[a]

Parameter/ Weekly Interval	Dose Groups (mg/kg/day)[b] 0	2.5	7.5	30.0	0	2.5	7.5	30.0
	Males				Females			
Serum glucose (mg/dL)								
Week 14	116±33.2	110±22.5	84±15.3*	79±6.0*	121±32.9	94±14.1*	97±25.6*	73± 8.8*
27	106±23.1	114±10.6	101±12.8	84±6.1*	111±14.9	100±16.6	107±25.5	85± 6.6*
52	116±27.3	122±27.9	104±13.0	97±13.0	107±7.9	114±18.8	103±22.1	91± 7.3*
92	105±28.0	120±34.3	102±21.9	89±14.2	107±32.9	96±21.3	107±19.6	95±20.9
Total protein (g/dL)								
Week 14	6.6±0.33	6.5±0.26	6.5±0.34	6.3±0.48	7.0±0.39	7.0±0.40	6.5±0.45*	6.0±0.29*
27	6.7±0.39	6.8±0.17	6.6±0.32	6.5±0.16	7.5±0.45	7.9±0.58	7.0±0.50	7.2±0.62
52	6.5±0.26	6.7±0.24	6.5±0.28	6.6±0.41	7.5±0.41	7.3±0.49	7.2±0.53	7.2±0.40
92	6.8±0.66	6.7±0.41	6.7±0.30	6.5±0.64	7.5±0.47	7.5±0.61	7.3±0.39	7.1±0.94
Calcium (mg/dL)								
Week 14	10.3±0.40	10.2±0.57	10.1±0.29	9.8±0.43	10.6±0.34	10.4±0.33	10.2±0.38	9.8±0.39*
27	11.0±0.61	11.1±0.42	10.7±0.36	10.4±0.35*	11.4±0.41	11.6±0.46	11.1±0.39	10.9±0.43
52	10.7±0.35	10.8±0.27	10.6±0.33	10.3±0.41	11.0±0.44	10.9±0.28	10.9±0.27	10.6±0.30
92	11.0±0.86	10.6±0.59	10.5±0.54	10.0±0.55*	11.0±0.48	10.9±0.37	10.7±0.25	10.3±0.78
Albumin (g/dL)								
Week 14	4.7±0.26	4.5±0.30	4.6±0.28	4.8±0.33	5.3±0.31	5.2±0.36	4.8±0.37*	4.6±0.28*
27	4.8±0.36	4.9±0.28	4.8±0.35	4.7±0.19	5.8±0.45	6.1±0.72	5.5±0.48	5.5±0.51
52	4.5±0.37	4.7±0.18	4.5±0.21	4.5±0.30	5.5±0.52	5.3±0.34	5.5±0.43	5.4±0.27
92	4.2±0.70	4.2±0.47	4.2±0.34	3.8±0.75	5.2±0.74	5.0±0.66	4.9±0.31	4.8±0.88

(continued)

TABLE 5 (continued)

Parameter/ Weekly Interval	Dose Groups (mg/kg/day)[b]							
	0	2.5	7.5	30.0	0	2.5	7.5	30.0
	Males				Females			
Globulin (g/dL)								
Week 14	1.9±0.22	2.0±0.29	1.9±0.23	1.5±0.49*	1.8±0.23	1.8±0.20	1.8±0.37	1.4±0.22*
27	1.9±0.23	1.9±0.24	1.8±0.24	1.8±0.26	1.7±0.25	1.8±0.29	1.6±0.27	1.6±0.28
52	2.1±0.30	2.0±0.11	1.9±0.25	2.1±0.40	2.0±0.36	1.9±0.33	1.7±0.45	1.8±0.35
92	2.7±0.50	2.5±0.34	2.5±0.30	2.8±0.78	2.3±0.47	2.5±0.60	2.4±0.33	2.4±0.49
Triiodothronine (ng/dL)								
Week 14	74.6±15.00	75.1±7.74	72.5±11.81	61.2±15.22	75.6±13.32	86.8±22.39	74.1±13.43	62.5±15.81
27	72.3±18.71	83.6±12.92	72.3±15.31	63.4±14.16	84.0±11.46	76.2±16.14	76.5±23.20	71.6±13.02
52	71.4±19.76	79.9±11.35	81.8±19.83	70.4±24.41	93.4±17.34	86.7±24.90	71.3±26.86	84.5±16.49
92	89.8±42.94	66.9±24.15	60.2±19.85*	44.9±19.82*	81.0±17.51	71.8±16.08	67.8±19.51	61.7±16.46*
Thyroxine (µg/dL)								
Week 14	5.5±1.39	5.9±0.79	5.6±0.86	3.5±1.14*	3.0±0.93	4.0±1.28	3.3±0.53	2.7±0.74
27	4.8±1.08	5.3±1.28	5.3±1.14	4.9±0.56	2.4±0.51	2.6±0.58	3.0±0.58	2.8±0.67
52	3.4±1.26	4.3±0.97	4.5±1.22	4.1±1.19	2.2±0.60	2.5±0.64	2.6±0.76	2.4±0.47
92	3.3±1.01	3.4±0.46	3.3±1.04	2.3±0.84*	2.4±0.69	2.3±0.80	2.5±0.42	2.1±0.49

[a]Data were extracted from Table 7 of the study report.

[b]Dose levels were lowered to 0, 1.0, 2.5, or 7.5 mg/kg/day at week 17

*Significantly different from control values, $p<0.05$.

calcium in high-dose females (7.5%), increased inorganic phosphorus in high-dose males (13%), increased gamma glutamyltransferase in high-dose males (200%) and females (100%), decreased sodium in high-dose males (1.4%) and increased potassium in high-dose males (11.7%).

Alterations in thyroid hormone levels were noted in the mid- and/or high-dose males and females. Significant decreases in thyroxine levels were observed in the high-dose males at weeks 14 (36%) and 92 (30%). Additionally, triiodothyronine levels were significantly decreased in the mid- and high-dose males (33% and 50%, respectively) and in high-dose females (24%) at week 92.

Although significant increases in total cholesterol levels (data not shown) were observed in all treated males at week 14, the increases were not dose related. Other clinical chemistry changes were sporadic and not considered to be treatment-related.

(c) Urinalysis

Urinalyses were performed on the first 10 animals/sex/group at weeks 14, 27, 52, 79, and at termination. The checked (x) parameters were examined.

X Appearance*	X Sediment (microscopic)	X Bilirubin*
X Volume*	X Protein*	X Blood
X Specific gravity*	X Glucose*	Nitrate
X pH*	X Ketones	X Urobilinogen

* = Recommended by Subdivision F (November 1984) Guidelines

Results:

Summary tabulation of urinalysis data was not provided; however, individual data were provided. An increase in the incidence of urinary ketone bodies was noted in the mid- and high-dose rats at week 14; in the high-dose rats at weeks 27, 52 and 79; and in the mid- and high-dose males and high-dose females at week 92 (results summarized in Table 6). The increase in urinary ketone bodies may have been partially due to decreased nutritional status of the animals.

5. Sacrifice and Pathology

Necropsy was performed on all animals. Tissues were preserved in 10% neutral-buffered formalin. At termination, a thorough histopathological evaluation was performed on all control and high-dose animals, and on all animals that died or were sacrificed in a moribund condition during the study. In addition, the thyroid gland from all animals was examined microscopically. Also, the lungs, livers, and kidneys in the low- and mid-dose animals were examined microscopically. The tissues checked (X) below were examined

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TABLE 6. The Incidence of Urinary Ketone Bodies at Selected Weeks for Rats Dosed With Aqueous Hydrogen Cyanamide for 91 weeks[a]

	Dose Level (mg/kg)[a,b]			
Weeks	0	2.5	7.5	30.0
		Males		
14	6/10[c]	4/10	10/10	10/10
27	5/10	5/10	5/10	9/9
52	3/9	5/10	5/10	5/9
79	1/10	2/10	1/11	5/10
92	3/9	6/13	9/14	12/14
		Females		
14	0/10	1/10	6/10	10/10
27	0/10	0/10	0/10	6/7
52	0/10	2/10	2/10	6/7
79	0/11	0/10	2/10	9/10
92	1/16	4/13	2/8	8/14

[a]Data were extracted from Appendix 9 of the study report.[a,c]
[b]Dose levels were lowered to 0, 1, 2.5, or 7.5 mg/kg/day[a,c] at week 17
[c]Number in the denominator indicates total number of animals examined

histologically. In addition, the double-checked (XX) organs were weighed from 10 rats/sex/group sacrificed at termination.

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain*
X Salivary glands*	X Heart*	X Peripheral nerve (sciatic nerve)*
X Esophagus*	X Bone marrow*	X Spinal cord* (three levels)
X Stomach*	X Lymph nodes*	X Pituitary*
X Duodenum*	X Spleen*	X Eyes (optic nerve)*
X Jejunum*	X Thymus*	
X Ileum*		Glandular
X Cecum*	Urogenital	
X Colon*		XX Adrenals*
X Rectum*	XX Kidneys*	Lacrimal gland
XX Liver*	X Urinary bladder*	X Mammary gland*
Gallbladder*	XX Testes*	X Thyroids*[a]
X Pancreas*	X Epididymides	X Parathyroids*[a]
	X Prostate	Harderian glands
Respiratory	X Seminal vesicle	
	XX Ovaries	
X Trachea*	X Uterus*	
X Lung*	X Vagina	
	X Cervix	

Other

X Bone (sternum and femur)*
X Skeletal muscle*
X Skin*
X All gross lesions and masses*

* = Recommended by Subdivision F (November 1984) Guidelines

[a] Weighed postfixation

(a) Organ weights:

A slight (6.7%) but statistically significant ($p \leq 0.05$) decrease in absolute brain weight was noted in the high-dose females when compared to controls. Statistically significant increases in relative (to body weight) organ weights were observed in the high-dose males and/or females; these included the thyroid (with parathyroid), brain, kidney, liver and testis with epididymides. However, the increases in relative organ weights may have been due to the significant decreases in mean terminal body weights in the high-dose animals.

(b) Gross pathology:

No gross lesions attributable to administration of the test material were found.

(c) Microscopic pathology:

The only treatment-related nonneoplastic lesion was confined to the thyroid gland. An increased incidence of slight-to-moderate reduced colloid, characterized by microfollicles, was noted in the mid- and high-dose animals. Also, this lesion was observed in animals at both scheduled and unscheduled deaths. The incidences of reduced colloid in the thyroid (combining incidences at unscheduled and scheduled deaths) for the control, low-, mid-, and high-dose males were 0/20, 0/18, 7/20, and 17/18, respectively. In females, the incidences were 3/20, 1/20, 5/20, and 16/20, respectively.

No increase in the incidence of neoplastic lesions in dosed groups were observed.

D. DISCUSSION

The design and conduct of this chronic oral study in rats were adequate. Initially this study was intended to be 52 weeks in duration, but because of concern regarding the potential of test material residue on the intended-use crop, the sponsor requested that the study be extended to 104 weeks. However, the study was terminated at week 92 due to low survival in the mid-dose females. In addition, dose levels were lowered at week 17 as a consequence of the marked reductions in body weight during the early part of the study.

The results of this study indicate the thyroid as a possible target organ for hydrogen cyanamide toxicity. An increase in the incidence of reduced colloid was noted in the mid- and high-dose animals. In addition, the analysis of thyroid hormone levels revealed possible treatment-related effects on thyroid function as indicated by decreases in both thyroxine and triiodothyronine levels in males receiving 7.5 mg/kg/day.[a,c] Although a previous mouse oncogenicity study (cited in the study report as Hazleton Project No. 55613) identified the ovary as a potential target organ, there were no treatment-related ovarian lesions noted in this rat study.

Significant reductions in body-weight gain were observed in both mid- and high-dose males and females, especially during the first 16 weeks of the study. A slight but statistically nonsignificant decrease in food consumption was noted in the high-dose males and females during the first 16 weeks of the study.

Numerous differences from control rats were noted in clinical pathology parameters, particularly at week 14. Results of the 14-week hematological analyses suggested anemia (decreased red blood cell count, hemoglobin and hematocrit levels) only in the high-dose males; these effects were not significant following week 14, possibly reflecting the lowering of dose levels at week 17. Increases in lymphocyte count were seen in mid- and high-dose males and females at week 14. Platelet counts were decreased in mid- and high-dose males

and females at weeks 14 and 27, but were still within the normal range. Among the clinical chemistry parameters observed in both mid- and/or high-dose animals at week 14, there were significant decreases in glucose and globulin levels, and increases in GGT activity.

Based on significant decreases in body weight gains and an increased incidence of reduced colloid in the thyroid, the LOEL is 7.5 mg/kg/day active ingredient hydrogen cyanamide in males and females. The NOEL is 2.5 mg/kg/day active ingredient hydrogen cyanamide for both sexes.

010591

FINAL

DATA EVALUATION REPORT

AQUEOUS HYDROGEN CYANAMIDE

Study Type: Reproductive Toxicity

Prepared for:

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031

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Independent Reviewer	Pia Lindström, DPH	Date 8/13/93
QA/QC Manager	Sharon Segal, Ph.D.	Date 8/13/93

Contract Number: 68D10075
Work Assignment Number: 2-78
Clement Number: 205
Project Officer: Caroline Gordon

EPA Reviewer: Ann Clevenger, Ph.D.
Review Section I, Toxicology Branch I/HED

Signature: Ann Clevenger (for)
Date: 8/17/93

EPA Section Head: Marion Copley, D.V.M.
Review Section IV, Toxicology Branch I/HED

Signature: Marion Copley
Date: 8/19/93

DATA EVALUATION REPORT

010591

STUDY TYPE: Reproductive toxicity; Guideline Series 83-4

EPA IDENTIFICATION NUMBERS

PC CODE: 014002

TOX CHEM. NUMBER: ~~260~~ 140

MRID NUMBER: 415665-04

TEST MATERIAL: Aqueous hydrogen cyanamide (50% w/w)

SYNONYM: None

SPONSOR: SKW Trostberg, Trostberg, Germany

STUDY NUMBER: 2319-126

TESTING FACILITY: Hazleton Laboratories America, Inc., Vienna, VA

TITLE OF REPORT: Two-Generation Reproduction Study in Rats with Aqueous Hydrogen Cyanamide (50% w/w)

AUTHOR: Sandra L. Morseth

REPORT ISSUED: April 19, 1990

CONCLUSIONS: In a two-generation reproduction study, Crl:CD BR rats were fed aqueous hydrogen cyanamide (50% w/w) daily by gavage at dose levels of 0, 2.5, 7.5, or 30 mg/kg/day a.i. (F_0 generation during premating) or 0, 1.25, 3.75, or 15 mg/kg/day a.i. during premating (F_1 generation), gestation and lactation (F_0 and F_1 females).

Parental NOEL = 1.25 mg/kg/day a.i.
Parental LOEL = 3.75 mg/kg/day a.i. based on significant decreases in body weight/weight gain and food consumption

Reproductive NOEL = not determined
Reproductive LOEL = 1.25 mg/kg/day a.i. based on decreased pup viability and body weight in both generations

In addition, at ≥ 15 mg/kg/day a.i., decreased fertility was observed in both generations.

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CLASSIFICATION: CORE Minimum Data.
Even Though there is NO Reproduct. NOEL, a new study is not required,
This study does satisfy the guideline requirement for a reproduction study (83-4) in rats.

SPECIAL REVIEW CRITERIA: (40 CFR 154.7) None

A. MATERIALS

Test Compound

Purity:	50% w/w
Description:	Clear colorless liquid
Lot number:	07/07/87
Date received:	July 29, 1987
Contaminants:	None reported
Storage:	Under refrigeration
Expiration date:	Not reported
Vehicle:	Distilled water (lot numbers: 222901; 0826903; 1028901; and 1229902)

Test Animals

Species:	Rat
Strain:	Crl:CD®BR (VAF/Plus™)
Source:	Charles River Laboratories, Inc., Raleigh, NC
Age:	≈7 Weeks at dose initiation
Weight:	F_0 males--181-315 g at study initiation F_0 females--161-205 g at study initiation

B. STUDY DESIGN

This study was designed to assess the potential of aqueous hydrogen cyanamide to cause reproductive toxicity when administered daily by gavage for two successive generations in rats.

Mating: After 21 days of acclimatization followed by approximately 14 weeks of daily oral administration, F_0 females were paired with males in a ratio of 1:1 until a plug or sperm was detected in a vaginal smear (or for a maximum of 21 days). The day of observation of sperm or plug was designated as day 0 of gestation (GD).

After at least 14 weeks of treatment, the F_1 parental animals were paired in a similar manner for a maximum of 21 days; sibling matings were avoided.

Animal Husbandry: Food (Purina® Certified Rodent Chow #5002) and tap water were supplied ad libitum. Temperature and humidity were maintained at 67-85°F and 24-85%, respectively. A 12/12-hour light/dark cycle was maintained; frequency of air changes was not reported.

Group arrangement: F_0 and F_1 animals were distributed using a computer-generated weight randomization procedure. F_1 animals were selected by random card draw. The animals were assigned to four groups as follows:

Test Group	Dietary Level in mg/kg/day* a.i.	Number Assigned per Group F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	26	26	26	26
Low dose	1.2 5	26	26	26	26
Mid dose	3.7 5	26	26	26	26
High dose	15	26	26	26	26

*F_0 parental animals received 0, 2.5, 7.5 or 30 mg/kg/day a.i. until the beginning of premating week 12.

Dose administered: The test solution was administered daily by gavage (10 mL/kg/day) at dose levels of 0, 2.5, 7.5, or 30 mg/kg/day a.i. Because of severe weight loss in the F_0 animals from the high-dose group, the dose levels were amended during premating week 12 to be 0, 1.2 5, 3.7 5, and 15 mg/kg/day a.i. These doses were administered to the animals from then on, for two consecutive generations.

Test solutions were prepared weekly and stored under refrigeration. The desired amount of aqueous hydrogen cyanamide (50% w/w) was weighed and poured into a beaker. Distilled water was added to the beaker and the solution was mixed on a magnetic stirrer for 2-3 minutes. The content was poured into a precalibrated beaker; the beaker was rinsed with water and its content was poured into the precalibrated beaker. The desired volume of the test solution was achieved by adding distilled water; the solution was mixed on a magnetic stirrer and transferred to a jar for dosing. Analysis for concentration was performed by UV spectrophotometry weekly for the first 4 weeks and every 4 weeks thereafter. Analysis for stability at 2.5- and 30-mg/kg/day a.i. dose levels was conducted following 10 days of refrigeration. In addition, the test material stability at 2 mg/kg/day a.i. was analyzed after 7 days of refrigeration.

Dose rationale: A rationale for the selection of dose levels was not provided.

Observations: Observations for mortality and moribundity were conducted twice daily and cageside observation data were recorded once daily. Overall clinical examinations, body weight, and food consumption data were recorded weekly for all animals; and for females on GDs 0, 7, 14, and 20 and on lactation days 0, 4, 7, 14, and 21 with the exception of food consumption data, which was not recorded during mating or beyond lactation day 14.

The following data were recorded for each litter:

- Gestation length

- Number of live and dead pups, sex, clinical observations, and pup weight at birth and on lactation days 1, 4, 7, 14, and 21

- Gross and behavioral abnormalities

Uteri of mated females, found dead or sacrificed when moribund, were examined for implantation sites, early or late resorptions, and for live fetuses; ovaries were examined for corpora lutea. Uteri of apparently nonpregnant females were examined by pressing between two glass slides to detect implantation sites.

On lactation day 4, pups were randomly culled to 4/sex/litter whenever possible. Culled pups and pups dying or killed during lactation were examined externally then sacrificed and examined for visceral abnormalities. At weaning of the F_1 pups, 2 pups/sex/litter were selected randomly as potential F_1 parental animals. Of these, one pair was designated as the breeder and the second pair was designated as the alternate. All F_1 pups not selected for the F_1 parental group or for histopathological examination were sacrificed and subjected to gross examination.

Parental animals of both generations and 1 pup/sex/generation/group were sacrificed and subject to a complete necropsy after weaning including external surface; all orifices; cranial cavity; and cervical, thoracic, and abdominal viscera. The following tissues were preserved in 10% neutral buffered formalin. Histopathology was conducted on the following organs from the control and high-dose groups.

- Ovaries
- Uterus
- Vagina
- Gross lesions
- Testes
- Epididymides
- Seminal vesicles
- Prostate gland

Statistical analysis: The following analyses were conducted.

- Body weight, body weight change, food consumption, gestation length, and litter data (total number of pups delivered, and number of live pups during certain intervals of lactation period)--Levene's test, ANOVA and Dunnett's test

- Pup body weight-- ANCOVA and Dunnett's test (litter size as covariate)

- Reproductive indices, and pup and litter incidences-- Cochran-Armitage trend test, Chi-Square test, and Fisher-Irwin two-sample exact test

Compliance:

- A signed Statement of No Data Confidentiality Claim, dated April 30, 1990, was provided.

- A signed Statement of Compliance with EPA GLPs, dated April 19, 1990, was provided.

- A signed Quality Assurance Statement, dated April 18, 1990, was provided.

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C. RESULTS

Test Material Analysis

Concentration analysis revealed values from 94% to 103% of target. Based on the results of a previous study (HLA No.2319-125), the study author claimed that the test material was stable for 10 days stored under refrigeration, but the results were not provided. The stability analyses of aqueous cyanamide solution (49% w/w) conducted in another study (V91.391; MRID No. 421784-06) revealed a value of 99% demonstrating that the compound was stable for one week (when stored daily at 4°C for 22 hours and at room temperature for 2 hours).

Parental Toxicity

Mortality: No compound related mortalities were observed in either sex or generation. Incidental deaths/moribund sacrifices are described below.

In the F_0 generation, one male from the control group died of gavage error following dosing during week 5 of premating. One nonpregnant female at 15 mg/kg/day a.i. was found dead during the rest phase (week 6 postweaning); this female had thickened uterine walls, distended cervix, hydrometra, and fluid filled abdominal cavity. The cause of death was not determined.

In the F_1 generation, one male at 3.75 mg/kg/day a.i. died during the F_2 weaning phase; necropsy did not reveal any remarkable findings. One female each from the control and 1.25 mg/kg/day a.i. dose groups were sacrificed moribund on days 22 and 26 of gestation, respectively; these females did not deliver pups. In addition, one female from the control group was sacrificed moribund during week 1 of the rest period because of a perforated esophagus due to gavage error.

Clinical observations: Compound related clinical signs were observed at 30 mg/kg/day a.i. in F_0 males following week 8 of the premating and persisted until final sacrifice. Signs (data not shown) consisted of rough haircoat (12/26 males) and thin appearance (6/26 males); when the dose was reduced to 15 mg/kg/day a.i., these signs were not observed in F_1 males. Similar signs were seen in 3/26 F_0 females from the 30 mg/kg/day a.i. dose group. Incidental findings, noted in all dose groups including controls, consisted of chromodacryorrhea, alopecia, and cut teeth.

Body weight: Compound related effects on body weight and body weight gain were observed in both sexes and generations at mid- and high-dose levels (7/3.75 and 30/15 mg/kg/day a.i., respectively). Summaries of body weight and weight gain data for selected intervals are presented in Tables 1 and 2. Overall results are discussed below.

Among F_0 females, food consumption was decreased during weeks 0-12 of premating. It was comparable among all dose groups during gestation but was lower than control at the high-dose on lactation days 0-14 (data not shown).

In the F_1 generation among males, a significant decrease in food consumption was noted at high-dose during weeks 0-23 of premating (Table 3), mating, and postmating periods except for weeks 1-4, 7-8, and 18-19.

Among F_1 females, food consumption was decreased during weeks 0-14 of premating except for weeks 1-3, 6-8, and 12-13. Food consumption was lower than control at high-dose during the entire gestation period (days 0-20), but was comparable in all dose groups during the lactation days 0-14 (data not shown).

Gross/histopathology: No compound related gross/histopathological findings were observed in either sex or generation. Incidental histopathological findings occurring in the control and high-dose males in both generations consisted of mononuclear cells in epididymis, chronic inflammation of prostate, degeneration of testis and vacuolization and necrosis of the liver (F_0 males only).

Reproductive Toxicity

Compound related reproductive toxicity was observed at all dose levels. It was manifested as reduced fertility at the high-dose and significantly reduced viability and body weights of F_1 pups at all three dose levels. Summaries of these effects are presented in Tables 4 and 5. Detailed results are presented below.

In the F_0 generation (Table 4), a statistically nonsignificant decrease in fertility was observed at the high-dose and 3/20 dams failed to deliver their litters. The viability index was significantly lower than control in all dose groups. The number of live pups/litter on day 4 precull and day 7 postpartum at the high-dose was significantly lower than control. Pup body weight (both sexes) was considerably decreased at all dose levels by the time of weaning on day 21. It was significantly lower than control at the high-dose on day(s) 0 (females), 14 (males), and 21 (both sexes) postpartum; at the mid-dose on day 4 precull and postcull (both sexes), days 7-21 (both sexes); and at the low-dose on day 7 (males) postpartum.

In the F_1 generation (Table 5), a statistically nonsignificant decrease in fertility was observed at the high-dose. The viability index was significantly decreased compared to control in all dose groups in a dose-related manner. Incidental increases were noted in the mean number of live pups/litter at mid-dose on day 0 and in the lactation index at the high-dose. F_2 pup body weight was comparable in all dose groups.

No compound related clinical signs or gross/histopathological findings were observed in any litter or generation.

In the F_0 generation among males, body weight was significantly lower (4-32%; p≤0.05; Table 1) than control at the mid-dose during weeks 7-13 of premating; and at high-dose during weeks 1-24 of premating, mating, and postmating periods. Body weight gain for these males was significantly lower (p≤0.05; 83% at mid-dose and 40% at high-dose) on weeks 0-12; and was significantly higher (p≤0.05; 56% at mid-dose and 46% at high-dose) on weeks 12-24. Sporadic changes in body weight and weight gain at the low-dose were not considered to be compound related.

Among F_0 females, body weight was significantly lower (5-31%; p≤0.05); Table 1) than control at the mid-dose on weeks 4-13 of premating and week 16 during mating; and at high-dose on weeks 2-18 of premating and mating periods. Body weight gain was significantly lower (18-54%; p≤0.05) at the mid- and high-doses on weeks 0-12 of premating period. For the entire gestation period (GDs 0-20) at the high-dose, body weight (significantly) and weight gain (non-significantly) were lower (13-18%; Table 2) than control. However, the decrease in body weight gain during GDs 14-20 (35%) was partially attributed to a loss of three females. During lactation, body weight of these females was significantly lower (10-13%; Table 2) on days 0-7. Dams gained 25 g compared to a loss of 1 g in the control group during days 0-21. During the rest period, body weight and weight gain for high-dose females were significantly lower (≥11%; p≤0.05) on weeks 4-8 and 1-8, respectively (data not shown).

In the F_1 generation among males, body weight was significantly lower (6-25%; p≤0.05; Table 1) than control at the mid-dose on weeks 1, 13, and 14 of premating; and on weeks 18-21 and 24-27 of postmating period; and at the high-dose on weeks 0-27 during the premating, mating, and postmating periods. Body weight gain was significantly lower (15-67%; Table 1) at the mid-dose during weeks 8-27 and at high-dose on weeks 0-27 during the premating, mating, and postmating. Sporadic decreases in body weight and body weight gain in the low- and mid-dose groups were not considered to be compound related.

Among F_1 females, body weight was significantly lower (9-19%; p≤0.05; Table 1) than control at the high-dose on weeks 0-16 during the premating and mating periods. Body weight gain for these females was significantly lower (9%; p≤0.05; Table 1) on weeks 0-12 of premating period. For the entire gestation period (GDs 0-20), body weight and weight gain at the high-dose were lower (15-21%; p≤0.05; Table 2) than controls. During lactation period at the high-dose, body weight was lower (10-17%; p≤0.05; Table 2) on days 0-21, but body weight gain was higher for the same period. During the rest period, body weight at the high-dose was lower (9-16%; p≤0.01) than control on weeks 2-7; whereas high-dose dams lost 9.4 g between weeks 4-7 compared to a 1.6 g gain for controls.

Food consumption: Compound related effects were observed in food consumption (g/animal/week) during premating in high-dose F_0 and F_1 males and females. Sporadic changes in food consumption at low- and mid-dose levels were not considered to be compound related. Summary of results of food consumption for the selected intervals is presented in Table 3.

In the F_0 generation among males, a significant decrease in food consumption was noted at the high-dose during weeks 0-23 of premating (Table 3), mating, and postmating periods except for weeks 20-21.

D. REVIEWERS' DISCUSSION/CONCLUSIONS

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Test Material Analyses

Concentrations of the test material in the dosing solutions were within ±10% of nominal values. The stability of the test material in the dosing solution had previously been confirmed.

Parental Toxicity

Compound related parental toxicity was observed at the mid- and high-dose levels in both sexes and generations. It was manifested as significantly decreased body weight/weight gain (mid- and high-dose levels) and food consumption (high-dose only). No compound related effects were seen in mortality, clinical signs, or gross and microscopic observations.

Based on these results, the NOEL for parental toxicity was 1.5 mg/kg/day a.i.; the LOEL was 7.5 mg/kg/day a.i.

Reproductive Toxicity

Compound related reproductive toxicity was observed in both generations at all three dose levels. It was manifested as decreased (albeit statistically nonsignificant) fertility in both generations at high-dose; significantly decreased viability in both generations at all dose levels; and significantly decreased F_1 pup body weights at all dose levels. The study author considers the decreased pup viability on days 0-4 to be normal variation. This conclusion was based on the laboratory's historical control range (1.1-39.8%; mean = 9.97%). The reviewers disagree with this conclusion based on the following reasons:

1. A range of 1-40% decreased pup viability on days 0-4 cannot be considered "normal." Therefore, the historical control data are questionable.

2. The consistent decreases across generations and dose groups cannot be considered incidental or normal variation.

3. The decreases were statistically significant when compared to controls.

The reviewers consider the decreased viability to be compound related. A review of the litters affected (Table 6) from day 0 to day 4 of lactation indicates that in both generations there is an increase (statistically significant) in the percentage of pups dying or missing (presumably cannibalized) and in the litters affected on the basis of sex or simply total litters affected in all treated groups as compared to controls. The lack of a dose-response in the F_0 generation is due to the lower number of dams present at the high-dose level. The decrease in viability is noted early in lactation and may be mediated through compound in the milk or through effects subsequent to *in utero* exposure.

The decreased F_1 pup body weight support the lack of establishment of a reproductive NOEL and may be explained by the higher parental compound

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intake during F_0 premating. When the dose was decreased, no effect was noted in F_2 pup body weights.

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Based on these results, the LOEL for reproductive toxicity was 1.2 5 mg/kg/day a.i; the NOEL was not determined. A new study is not required at this time since there was a good dose response for the Reproductive effect and the magnitude in the LDT was low.

E. CLASSIFICATION: CORE Minimum Data.

Parental toxicity NOEL = 1.25 mg/kg/day a.i.
Parental toxicity LOEL = 3.7 5 mg/kg/day a.i. based on decreased body weight/weight gain and food consumption

Reproductive toxicity NOEL = Not determined
Reproductive toxicity LOEL = 1.2 5 mg/kg/day a.i. based on decreased viability

F. RISK ASSESSMENT: Not applicable

Table 1. Body Weight (g ± S.D.) During the Premating Period for Rats Administered Aqueous Hydrogen Cyanamide by Gavage for Two Successive Generations[a,b]

Study Week	Dose Level (mg/kg/day a.i.) 0		2.5/1.25		7.5/3.75		30/15	
F_0 Males								
0	275	22	285	12	286	15	284	13
3	403	21	397	23	388	29	323	23**
7	492	27	489	33	463	40*	356	26**
12	545	32	534	43	510	46*	392	36**
Wt. Gain 0-12	271	38	249	39	224	38**	108	30**
F_0 Females								
0	184	11	186	10	187	10	187	11
3	243	20	244	16	235	14	213	12**
7	285	24	286	20	269	18**	232	15**
12	313	30	312	23	292	22**	246	19**
Wt. Gain 0-12	129	24	126	17	106	15**	59	16**
F_1 Males								
0	89	14	78	17*	76	16**	69	16**
3	261	31	244	39	241	34	229	42**
7	438	41	418	46	417	44	376	53**
12	550	50	523	47	517	51	460	60**
Wt. Gain 0-12	461	44	446	42	441	43	391	49**
F_1 Females								
0	81	9	77	14	70	15**	66	14**
3	185	13	187	21	179	24	169	23*
7	261	19	264	23	255	30	231	25**
12	302	29	309	25	293	35	269	28**
Wt. Gain 0-12	221	31	232	23	222	28	202	22*

[a]Data were extracted from Study No. 2319-126, pp. 69-75, and 133-139.

[b]The dose levels were amended during premating week 12 to be 0, 2.5, 3.7 5, and 15 mg/kg/day a.i.

*Significantly different from control (p 0.05)
**Significantly different from control (p 0.01)

Table 2. Body Weight (g ± S.D.) During Gestation and Lactation for Rats Administered Aqueous Hydrogen Cyanamide for Two Successive Generations[a]

Study Days	Dose Level (mg/kg/day a.i.) 0		1.25		3.75		15	
Gestation:								
F_0 Generation-F_1 Litters								
0	317	36	324	27	309	25	261	20**
7	350	35	359	26	344	28	295	19**
14	374	35	387	27	373	28	324	20**
20	447	39	467	36	448	30	372	41**
Wt. Gain 0-20	130	22	143	18	139	17	111	36
F_1 Generation-F_2 Litters								
0	309	30	314	23	300	34	262	24**
7	336	29	344	24	327	36	278	24**
14	363	31	373	27	352	34	299	26**
20	428	27	443	35	426	42	356	41**
Wt. Gain 0-20	119	16	129	27	126	31	94	31**
Lactation:								
F_0 Generation-F_1 Litters								
0	359	36	363	28	351	29	313	17**
4	357	30	363	27	350	31	314	17**
7	360	27	367	23	356	26	323	18**
14	359	26	365	20	364	20	342	18
21	353	17	360	16	354	30	338	17
Wt. Gain 0-21	-1	24	0.56	19	3	24	25	14**
F_1 Generation-F_2 Litters								
0	348	32	361	29	337	30	291	19**
4	346	30	355	24	338	30	298	23**
7	348	30	359	27	345	30	307	25**
14	364	27	369	26	360	30	326	27**
21	352	20	352	30	347	30	318	31**
Wt. Gain 0-21	1	24	-8	22	7	18	27	18**

[a]Data were extracted from Study No. 2319-126, pp. 76-79 and 140-143.

**Significantly different from control (p 0.01)

Table 3. Food Consumption (g/animal/week) During the Premating Period for Rats Administered Aqueous Hydrogen Cyanamide for Two Successive Generations[a,b]

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Study Week	Dose Level (mg/kg/day a.i.) 0		2.5/1.25		7.5/3.75		30/15	
F_0 Males								
1 - 2	179	11	186	14	173	15	135	12**
2 - 3	182	13	189	15	172	14*	126	15**
5 - 6	182	11	189	15	180	18	143	14**
6 - 7	181	11	185	15	177	18	136	12**
10 -11	176	14	186	17	186	21	154	21**
11 -12	179	10	177	16	174	22	165	17**
F_0 Females								
1 - 2	132	15	135	18	129	18	114	11**
2 - 3	139	18	138	11	129	12*	112	12**
5 - 6	140	15	149	11*	136	14	112	12**
6 - 7	135	15	144	19	131	14	103	11**
10 -11	137	13	136	13	127	11*	105	11**
11 -12	128	14	134	12	125	12	112	12**
F_1 Males								
1 - 2	154	16	149	22	146	20	148	26
2 - 3	173	19	168	22	166	17	166	21
5 - 6	202	18	196	15	200	21	185	20**
6 - 7	201	18	204	18	200	21	187	20*
10 -11	209	19	206	14	201	25	180	17**
11 -12	210	19	208	24	201	29	175	18**
F_1 Females								
1 - 2	125	13	133	14**	131	13	124	18
2 - 3	133	12	146	16**	135	12	132	14
5 - 6	150	12	153	13	151	16	137	11**
6 - 7	145	13	155	27	147	14	138	21
10 -11	135	16	142	13	139	15	126	12*
11 -12	144	15	143	14	139	17	131	12**

[a]Data were extracted from Study No. 2319-126, pp. 82-86 and 146-150.

[b]The dose levels were amended during the premating week 12 to be 0, 1.25, 3.75 and 15 mg/kg/day a.i.

*Significantly different from control (p 0.05)
**Significantly different from control (p 0.01)

Table 4. Effects of Dietary Administration of Aqueous Hydrogen Cyanamide on F_0 Reproductive Parameters and F_1 Offspring Survival and Body Weight[a]

Parameter	Dose Level (mg/kg/day a.i.)			
	0	1.25	3.75	15
No. paired (F_0 parents)	26	26	26	26
No. positive matings	26	22	24	23
Mating index (%)[b]	100	85	92	88
No. Pregnant females	20	20	20	15
Female fertility index (%)[c]	77	91	83	65
Gestation index (%)[d]	100	100	100	80
Gestation length (days)	21.9	22.1	21.9	22.1
No. females with liveborn pups	20	20	20	12
Total no. live pups				
Day 0	257	295	288	128
Day 4 precull	247	252	258	100
Day 21	136	127	133	68
Mean no. live pups/litter with live pups				
Day 0	12.9	14.8	14.4	10.7
Day 4 precull	13.0 (19)	13.3 (19)	12.9	8.3**
Day 21	7.2 (19)	7.1 (18)	6.7	5.7
Live birth index (%)[e]	98	96	96	99
Viability index (%)[f]	92	83**	88**	84**
Lactation index (%)[g]	92	85	87	88
Mean pup body weight (g)				
Day 0				
males	6.7	6.6	6.5	6.3
females	6.3	6.3	6.1	5.9*
Day 7				
males	15.2	12.9*	12.4**	12.8
females	14.4	12.8	11.9**	12.9
Day 21				
males	50.5	47.1	43.2*	42.6*
females	49.0	47.0	40.4**	42.3*
Sex ratio (% males day 0)	43	55	53	56

[a]Data were extracted from Study No. 2319-126, pp 91-97 and 323-331.

[b]Mating index: No. of mated females expressed as % of No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]Live birth index: Percentage of pups born alive based on No. of total pups born

[f]Viability index: Percentage of pups surviving four days based on No. of pups on day 0

[g]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

*Significantly different from control (p 0.05)
**Significantly different from control (p 0.01)

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Table 5. Effects of Dietary Administration of Aqueous Hydrogen Cyanamide on F_1 Reproductive Parameters and F_2 Offspring Survival and Body Weight[a]

Parameter	Dose Level (mg/kg/day a.i.)			
	0	1.25	3.75	15
No. paired (F_0 parents)	26	26	26	26
No. positive matings	25	24	26	23
Mating index (%)[b]	96	92	100	88
No. Pregnant females	24	21	23	19
Female fertility index (%)[c]	96	88	88	83
Gestation index (%)[d]	96	95	91	89
Gestation length (days)	21.8	21.9	21.9	22.0
No. females with liveborn pups	23	20	21	17
Total no. live pups				
Day 0	291	263	309	196
Day 4 precull	268	224	255	157
Day 21	158	128	133	115
Mean no. live pups/litter with live pups				
Day 0	12.7	13.2	14.7*	11.5
Day 4 precull	11.7	11.2	12.8 (20)	9.2
Day 21	7.2 (22)	7.1 (18)	7.0 (19)	6.8
Live birth index (%)[e]	98	98	98**	95**
Viability index (%)[f]	93	87*	82**	81**
Lactation index (%)[g]	87	81	87	97*
Mean pup body weight (g)				
Day 0				
male	6.4	6.6	6.6	6.2
female	6.0	6.1	6.2	5.8
Day 7				
male	13.4	12.6	13.1	13.0
female	12.7	12.2	12.3	12.3
Day 21				
male	44.8	43.1	43.8	42.7
female	43.2	42.7	41.1	40.0
Sex ratio (% males day 0)	56	44	48	49

[a]Data were extracted from Study No. 2319-126, pp 154-160 and 749-756.

[b]Mating index: No. of mated females expressed as % of No. of paired females

[c]Fertility index: No. of pregnant females expressed as % of No. of paired females

[d]Gestation index: No. of females delivering a live litter expressed as % of No. of pregnant females

[e]Live birth index: Percentage of pups born alive based on No. of total pups born

[f]Viability index: Percentage of pups surviving four days based on No. of pups on day 0

[g]Lactation index: Percentage of pups surviving 21 days based on No. of pups on day 4 postcull

*Significantly different from control (p 0.05)
**Significantly different from control (p 0.01)

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Table 6. Pup viability in F_1 and F_2 Litters in Rats Administered Aqueous Hydrogen Cyanamide by Gavage for Two Successive Generations[a]

Group	Dose Level (mg/kg/day a.i.)			
	0	1.25	3.75	15
F_1 Generation				
Males	2	9	9	6
Females	6	7	7	5
Total litters affected on sex basis	8	16	16	11
Total litters affected	8	9	11	6
(number affected/total number available)	(40%)	(45%)	(55%)	(50%)
F_2 Generation				
Males	4	4	9	10
Females	4	7	7	8
Total litters affected on sex basis	8	11	16	18
Total litters affected	5	8	11	12
(number affected/total number available)	(22%)	(40%)	(52%)	(71%)

[a]Data were compiled by the reviewers using Appendix 15.

FINAL

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DATA EVALUATION REPORT

Hydrogen Cyanamide

Study Type: Metabolism (Literarure Review)

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Review Section IV, Toxicology Branch I, HED Date: 8/17/93

DATA EVALUATION REPORT

010591

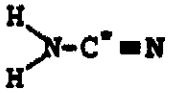
STUDY TYPE: Metabolism

TEST MATERIAL: Hydrogen cyanamide

EPA IDENTIFICATION NUMBERS:

PC Code: 014002
Tox. Chem. No.: ~~2665~~ 140
MRID Number: 421784-07

CHEMICAL STRUCTURE:



(*denotes position of radiolabel)

SPONSOR: SKW TROSTBERG AG, Postfach 1262, D-8823 Trostberg, Federal Republic of Germany

TITLE OF REPORT: Metabolism of Hydrogen Cyanamide

AUTHOR: ENVIRON Corporation

STUDY COMPLETION: September 5, 1991

ABSTRACT: 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 CO_2. Following both oral and i.v. 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.

CONCLUSIONS: Hydrogen cyanamide, a potent aldehyde dehydrogenase inhibitor, is used in alcohol aversion therapy to treat chronic alcoholism. Hydrogen cyanamide blocks ethanol metabolism by inhibiting aldehyde dehydrogenase which then causes an increase in blood acetaldehyde levels. The metabolism of hydrogen cyanamide is summarized from information obtained from ten different studies (See Appendix A).

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 (Deitreich et al. 1976; Mertschenk et al. 1991; Obach et al. 1989; Shirota et al. 1984). 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 (Deitreich et al. 1976). Negligible amounts were excreted as expired CO_2 (Deitreich et al. 1976). Following both oral and i.v. dosing in dogs, 62%-83.1% of the dose was excreted in the urine within the first 24-27 hours (Shirota et al. 1984). Negligible amounts of radioactivity were excreted in the feces (Shirota et al. 1984). The major metabolite of cyanamide excreted in the urine of rats, rabbits, dogs and humans was identified as N-acetylcyanamide (Shirota et al. 1984; Mertschenk et al. 1991). Following both i.v. and oral dosing in dogs, approximately 87% of the radioactivity was excreted as acetylcyanamide in the urine during the first 27 hours postexposure (Shirota et al. 1984). Unchanged hydrogen cyanamide represented 11%-12% of the dose in the dog urine (Shirota et al. 1984). Both humans and rats excreted approximately 40%-45.6% of the dose in the urine as acetylcyanamide within 48 hours postdosing (Mertschenk et al. 1991).

Pharmacokinetic studies show that cyanamide is rapidly absorbed in rats and dogs following both oral and i.v. dosing (Obach et al. 1989). Following oral dosing plasma concentrations peaked after 5 minutes in rats and 30 minutes in dogs. Bioavailability was ≈68.7% in rats dosed with 2 mg/kg and ≈65% in dogs dosed with 4 mg/kg. Following i.v. dosing, the elimination half-life in rats dosed with 2 mg/kg was 33 minutes, the elimination half-lives in dogs dosed with 1-4 mg/kg were dose dependent and ranged from 38.7 minutes to 61.3 minutes.

Dermal absorption studies in rats indicate that the amount of ^{14}C-hydrogen cyanamide absorbed was generally proportional to the dose and increased with time of exposure (LeVan 1989). The average ^{14}C equivalents absorbed within 24 hours were 2%-11% for doses ranging from 0.1 mg-10 mg ^{14}C-hydrogen cyanamide. Urinary excretion of radioactivity also increased with time and dose (0.93%-7.76% of the dose) after 24 hours of exposure for all dose groups. As in rats, dermal absorption of hydrogen cyanamide in humans is also limited (Mertschenk et al. 1991). Following application of hydrogen cyanamide (0.25 mg/kg) in humans, 2.3 mg of the dose was absorbed within 48 hours of exposure. The mean recovery of the dermally absorbed cyanamide was 7.7%.

Deitreich et al. (1976) demonstrated that hydrogen cyanamide does not inhibit rabbit or mouse liver aldehyde dehydrogenase in vitro, suggesting that a metabolite and not cyanamide is the inhibitor in vivo. This is supported by an in vitro study by DeMaster et al. (1982), using both yeast aldehyde dehydrogenase and rabbit skeletal muscle glyceraldehyde-3-phosphate dehydrogenase, which showed that mitochondria-catalyzed activation of cyanamide was necessary for aldehyde dehydrogenase inhibition. Shirota et al. (1984) demonstrated that 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.

Shirota et al. (1987a,b) demonstrated that hydrogen cyanamide is also metabolized in vitro to cyanide through an intermediate product hydroxycyanamide using rat liver microsomes; however, this has not been supported in vivo. There was no significant increase in blood cyanide

concentrations in humans orally exposed to hydrogen cyanamide (0.25 mg/kg), indicating that the metabolic degradation of hydrogen cyanamide to cyanamide is not a relevant pathway in humans. In addition, Shirota and Nagasawa 1988 demonstrated that this secondary pathway is not relevant for mice, rats or dogs in vivo. They examined this pathway using different species/strains of animals with comparatively low N-acetyltransferase activity, assuming that this secondary pathway might play a larger role in these animals. However, the results showed that the second metabolic pathway was of minor importance in vivo.

The metabolic pathways for hydrogen cyanamide are presented in Appendix A.

CLASSIFICATION: ~~Supplementary~~ Acceptable. Although the literature review provides data on the absorption, metabolism, and excretion of hydrogen cyanamide for various species and routes, these submitted data only partially fulfill the guideline requirements (85-1) for a general metabolism study. ~~manufacturer~~ The previous deficiencies do not warrant further metabolism testing at this time.

A. MATERIALS and METHODS

1. Test Material

Animal Studies

The test material used in all of the animal studies was pure chemical grade cyanamide. In all studies, the parent compound alone was administered to the animals. Radiolabeled cyanamide was used by Deitrich et al. (1976) (International Chemical and Nuclear Corporation), Shirota et al. (1984) (P-L Biochemicals, Milwaukee, WI), and Shirota and Nagasawa (1988) (ICN Pharmaceuticals, Irvine, CA). Nonradiolabeled cyanamide was used by Mertschenk et al. (1991) (SKW Trotsberg AG, Trostberg, FRG) and Obach et al. (1989) (Fluka, Switzerland). LeVan (1991) used both radiolabeled (radiopurity of 87%-90%; Amersham Corporation) and nonradiolabeled cyanamide.

In vitro studies:

In the in vitro studies, Deitrich et al. (1976), DeMaster et al. (1988), and Shirota et al (1984), (1987a), and (1987b) used hydrogen cyanamide commercially purchased from Sigma Chemical Co., St. Louis, MO or Aldrich laboratories, Milwaukee, WI; DeMaster et al. (1982) did not report the origin of the test material.

Human studies:

Commercially prepared cyanamide was used. Shirota et al. (1984) used cyanamide as carbimide (200 mg in 20 ml of H_2O) in one subject and Dipsan (50 mg) in another. Cyanamide in the form of its citrated calcium salt (Temposil, Dipsan, Abstem) or as a 1% aqueous solution is available as an alcohol deterrent agent for the treatment of alcoholism in Canada, Europe and Japan. Mertschenk et al. (1991) used nonradiolabeled cyanamide obtained from SKW Trotsberg, Trotsberg, FRG.

2. Test Animals

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A summary of the various species used in the in vivo studies is presented in Table 1.

3. Dosing/Sample Collection

Animal studies

Deitreich et al. (1976): Male and female Sprague Dawley rats were injected intraperitoneally with 10 μCi/kg of ^{14}C-hydrogen cyanamide. Expired CO_2 and urine were collected 1, 4, and 6 hours postdosing.

LeVan (1989): A single dermal dose of 0.1, 1, or 10 mg ^{14}C-hydrogen cyanamide was administered to three groups of male rats (24/group).

TABLE 1. SUMMARY OF EXPERIMENTAL ANIMALS

Reference	Species and Strain Tested/Sex/Weight				
	Rats	Mice	Rabbits	Dogs	Man
Deitrich et al. (1976)	Sprague-Dawley/Males & Females/ 150-250 g				
Shirota et al. (1984)	Sprague-Dawley/Males/ 160-180 g		New Zealand White/Males/ 4 kg	Beagle/ Males/ 9-11 kg	Males
Shirota and Nagasawa (1988)	Sprague-Dawley	A/J		Beagle	
	WKYN/N	DBA/J			
Mertschenk et al. (1991)	Wistar/ Males/ 233 g				Males
Obach et al. (1989)	Sprague-Dawley/ Males/ 200-220 g			Beagle/ Males / 9-16 kg	
LeVan (1991)	Crl:CD(SD)BR/ Males/ 201-233 g				

Mertschenk et al. (1991): Four male Wistar rats (233 g, mean weight) were administered a gavage dose of 10 mg/kg hydrogen cyanamide. Urine was collected 48 hours postdosing and was analyzed for acetylcyanamide.

Obach et al. (1989): Rats (48/group) were each given an aqueous solution of 2 mg/kg cyanamide by either i.v. or gavage. Blood samples were obtained 1, 3, 10, 15, 20, 30, 45, 60, 90, and 120 minutes following i.v. dosing and 1, 5, 7, 10, 15, 20, 30, 45, 60, 90, and 120 minutes following oral dosing.

Five male beagle dogs were given i.v. doses of 1, 2, and 4 mg/kg hydrogen cyanamide (aqueous solution). Each dog received three single doses with an interval of one week between administrations. Dogs were also given a gavage dose of 4 mg/kg hydrogen cyanamide. Blood samples were drawn before administration and at different times up to 6 hours postdosing.

Shirota et al. (1984): Male Sprague-Dawley rats (160-180 g) were administered hydrogen cyanamide (1.0 mmol/kg, i.p.) at 0 and 48 hours. Male New Zealand White rabbits (4 kg) received hydrogen cyanamide dissolved in saline (1.75 mmol/kg, i.v.) via the marginal ear vein. Two male Beagle dogs (9-11 kg) were given an oral dose (0.04 mmol/kg, 12 and 15.1 μCi) or an i.v. dose (0.04 mmol/kg, 13.9 μCi) of ^{14}C-cyanamide. Urine samples were collected for a period of 0-12 hours for the rats and rabbits, and from 0-336 hours for the dogs.

Shirota and Nagasawa (1988): Mice, rats, and dogs were dosed (i.p.) with 75 mg/kg (4.57×10^6 dpm for A/J and DBA/J mice; 23.15×10^6 and 30.86×10^6 dpm for the WKY/N and SD rats, respectively); beagle dogs were dosed orally with 0.04 mmol/kg (19.26×10^6 dpm).

Human studies

Mertschenk et al. (1991): Male human volunteers were given an oral dose of 0.25 mg/kg hydrogen cyanamide in drinking water. Urine samples were collected over a period of 48 hours in 12-hour aliquots.

Shirota et al. (1984): An adult male Japanese patient was given a single oral dose of cyanamide (carbimide, 200 mg in 20 ml of H_2O). The urine was collected 8 hours postdosing. A second urine sample was collected from a male Caucasian patient (59 years of age) who was maintained on Dipsan (50 mg) twice daily for 4 years.

4. Test System

In Vitro Studies:

Deitrich et al. (1976) investigated inhibition of aldehyde dehydrogenase in brain and liver homogenates using a maximum concentration of 10^{-3} M cyanamide.

DeMaster et al. (1982) incubated yeast aldehyde dehydrogenase and cyanamide in the presence and absence of intact rat liver mitochondria. Liver mitochondria were isolated in 0.25 M sucrose-0.1 mM EDTA from male Sprague-Dawley rats (180-250 g). Yeast aldehyde dehydrogenase and rabbit skeletal muscle

glyceraldehyde-3-phosphate dehydrogenase were obtained from Sigma Chemical Company, St. Louis, MO. A stock solution of yeast aldehyde dehydrogenase containing 30% glycerol, 1 mM EDTA, and 0.1 M potassium phosphate buffer, pH 8 was prepared. The yeast enzyme and cyanamide (200 μM) were incubated in the presence and absence of rat liver mitochondria. The activity of glyceraldehyde-3-phosphate dehydrogenase was assayed by adding to a reaction mixture containing 0.15 mM NAD^+, 1 mM D,L-glyceraldehyde-3-phosphate, 5 mM sodium arsenate, 1.25 mM EDTA, and 50 mM Tris-Cl, (pH 8.6). The activity was measured by following the increase in absorbance at 340 nm (25°C).

DeMaster et al. (1988) substituted cumene hydroperoxide for hydrogen peroxide in the oxidation of cyanamide by bovine liver catalase. A complete incubation system was used which contained 4.2 mM cumene hydroperoxide or 10 mM glucose/50 μg glucose oxidase, 40 mM cyanamide, 2 mg catalase and 100 mM potassium phosphate buffer (pH 7). Incubations were carried out at 37°C and quenched with 0.5 mL concentrated phosphoric acid.

Shirota et al. (1984) isolated hepatic N-acetyltransferase from the rabbit and dog. Incubation mixtures were prepared containing acetyl-S-CoA (2 μmol), ^{14}C-cyanamide (0.4 μmol, 9.2×10^5 dpm), hepatic N-acetyltransferase (7.7 mg protein), and phosphate buffer (0.1 M, pH 6.8). The reaction was initiated by the addition of cyanamide or buffer. Incubations were carried out at 37°C for 20 or 40 minutes. The reaction was quenched by the addition of 1 ml of ethanol and cooled to 4°C.

Shirota et al. (1987a) isolated microsomes from the livers of male Sprague-Dawley rats (200-250 g). Cyanamide was obtained from Sigma Chemical Company, St. Louis, MO. Incubation mixtures were prepared which contained glucose-6-phosphate (2.5 mM), potassium chloride (16.5 mM), magnesium chloride (4 mM), cyanamide (40 mM), glucose-6-phosphate dehydrogenase (5 units), microsomes (uninduced or PB induced (5.65 mg protein), and when indicated SKF-525a (4 μM). The reactions were initiated by the addition of enzyme and quenched by the addition of 0.5 mL of concentrated phosphoric acid.

Shirota et al. (1987b) used cyanamide, bovine liver catalase, and glucose oxidase purchased from Sigma Chemical Co., St. Louis, MO. Incubation mixtures were prepared which contained glucose-6-phosphate (2.5 mM), potassium chloride (16.5 mM), magnesium chloride (4 mM), cyanamide (40 mM), glucose-6-phosphate dehydrogenase (5 units), microsomes (uninduced or PB induced (5.65 mg protein), and when indicated SKF-525a (4 μM). The reactions were initiated by the addition of enzyme and quenched by the addition of 0.5 mL of concentrated phosphoric acid.

B. RESULTS/CONCLUSIONS

Animal Studies (In vivo)

Deitreich et al. (1976): Following i.p. dosing of ^{14}C-cyanamide (10 μCi/kg), the ^{14}C label was rapidly excreted in the urine of rats. At the end of 6 hours, 93.9% of the dose appeared in the urine. Negligible amounts of ^{14}C were excreted as expired CO_2 (1.39% of the dose) 6 hours postdosing. The liver contained 6.7% of the dose at 1 hour postdosing, but no radioactivity was detected in the liver thereafter.

LeVan (1989): A single dermal dose of 0.1, 1, or 10 mg ^{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. Urinary excretion of radioactivity also increased with time and dose (0.93%-7.76% of the dose) after 24 hours of exposure for all dose groups.

Mertschenk et al. (1991): Following oral administration of hydrogen cyanamide (10 mg/kg) to rats, an average of 45.6% of the applied dose was excreted in the urine as acetylcyanamide within 48 hours (42.7% excreted within the first 22 hours).

Obach et al. (1989): Absorption of cyanamide was rapid in rats following oral administration (2 mg/kg). Plasma concentrations peaked after five minutes. Bioavailability was ≈68.7%. Following i.v. administration, plasma clearance was 117 ml $kg^{-1}min^{-1}$ and the elimination half-life was 33 minutes.

Oral absorption of cyanamide was rapid in dogs dosed with 4 mg/kg; plasma concentrations peaked 30 minutes after administration. Bioavailability of hydrogen cyanamide after oral administration of 4 mg/kg was ≈65%. The pharmacokinetic behavior of cyanamide following i.v. dosing (1, 2, or 4 mg/kg) was dose dependent. Plasma clearance decreased when the dose increased. Elimination half-lives ($T_{½}$) increased with dose. Elimination $T_{½}$ for the 1, 2, and 4 mg/kg groups were 38.7, 47.2, and 61.3 minutes, respectively.

Shirota et al. (1984): The structure of the major urinary metabolite of cyanamide in rats, rabbits, and dogs was determined to be N-acetyl-cyanamide. Thus, cyanamide is acetylated in vivo to N-acetylcyanamide. Excretion in dogs was rapid following both oral and i.v. dosing (0.04 mmol/kg). The urine was the major route of excretion. Following oral exposure the majority of the radioactivity appeared in the urine within the first 24-27 hours (67.5%-83.1% of the dose). The total amount of radioactivity excreted in the urine was 99.2% at 120 hours postdosing in dog 1 and 85.9% at 336 hours in a dog 2. Only small amounts of radioactivity (0.3%) were eliminated via the feces. No feces were excreted prior to 24 hours. Intravenous dosing of dog 2 revealed similar results, suggesting that the liver, not the gastrointestinal

tract, was the major site for its biotransformation. The majority of the dose was excreted in the urine (62%) within 24 hours. By 336 hours postdosing, 79.7% was excreted in the urine. A total of 86.9% of the urinary radioactivity excreted between 0 and 27 hours was present as N-acetylcyanamide in dogs following oral and i.v. dosing. Unchanged hydrogen cyanamide accounted for ≈11% of the urinary radioactivity.

Shirota and Nagasawa (1988): This study examined whether using animal species with comparatively low or minimal N-acetyltransferase activity might lead to a larger role for a minor metabolic pathway in which hydrogen cyanamide is metabolized to cyanide. (See metabolic pathway Appendix A.) This minor metabolic pathway involves catalase and/or cytochrome P_{450}-mediated oxidation of cyanamide to N-hydroxycyanamide which then decomposes to nitroxyl and cyanide. Cyanide is then metabolized to 2-iminothiozolidine-4-carboxylic acid (ITCA) and thiocyanate (SCN^-). The Sprague-Dawley rats and DBA/J mice represented normal animals, whereas WKY/N rats and AJ mice served as slow acetylator rodent species. The dog is generally known as a slow acetylator and cannot acetylate aromatic amines. The urine was collected at 24 hours and analyzed for cyanamide and its major metabolite acetylcyanamide, as well as ITCA and SCN^-. The normal acetylators excreted 62.4% (mice) and 70.3% (rats) of the radioactivity in the 0-24-hour urine, and the slow acetylators excreted 65% (A/J mice), 71.1% (WKY/N rats), and 70.5% (dogs) of the radioactivity in the 0-24-hour urine. The profile of the hydrogen cyanamide metabolites excreted in the urine was similar for both slow and normal acetylators of all species. The majority of the radioactivity was excreted as N-acetylcyanamide (50.3%-58% of the dose). The parent compound accounted for 2.6%-3% of the radioactivity in mice, 7.7%-8.6% in rats, and 8.6% in dogs. There was minimal incorporation of radioactivity into ITCA and SCN^-, indicating that only a small fraction of the dose was converted to the active aldehyde dehydrogenase inhibitor and cyanide in these animals. Cyanamide metabolism via the minor pathway, involving catalase and/or cytochrome P_{450} was not enhanced in the slow acetylator phenotypes. Thus, cyanide toxicity appears to be minimal.

Human Studies

Mertschenk et al. (1991): Male human volunteers were given an oral dose of 0.25 mg/kg hydrogen cyanamide in drinking water. Approximately 40% of the dose was excreted in the urine as acetylcyanamide within 48 hours (the majority excreted within the first 12 hours). Compared with control values, there was no significant increase in either blood cyanide concentrations or urine thiocyanate concentrations, demonstrating that hydrogen cyanamide is not metabolized to cyanide or thiocyanate in humans.

In a skin absorption study, a dermal application of hydrogen cyanamide (0.25 mg/kg) was given to the same group of volunteers (n=6) on a skin surface area of 32 cm^2. Within 48 hours of exposure, 2.3 mg of hydrogen cyanamide was absorbed. The mean recovery of maximum possible dermally absorbed cyanamide was 7.7%.

Shirota et al. (1984): An adult male Japanese patient was given a single oral dose of cyanamide (carbimide, 200 mg in 20 ml of H_2O). The

urine was collected 8 hours postdosing. A second urine sample was collected from a male Caucasian patient (59 years of age) who was maintained on Dipsan (50 mg) twice daily for 4 years. Cyanamide is the active component of the alcohol deterrent agent Dipsan. Analysis of the 8 hour urine sample indicates that man can also acetylate cyanamide.

In Vitro Studies

Deitreich et al. (1976): In vitro, hydrogen cyanamide (10^{-3} M) did not significantly inhibit rabbit liver aldehyde dehydrogenase or mouse liver aldehyde dehydrogenase, suggesting a metabolite is the inhibitor in vivo.

DeMaster et al. (1982): To determine if aldehyde dehydrogenase inhibition is dependent upon the conversion of cyanamide to an active form, yeast aldehyde dehydrogenase and cyanamide were incubated in the presence and absence of intact rat liver mitochondria. Mitochondria-catalyzed activation of cyanamide was also tested using rabbit skeletal muscle glyceraldehyde-3-phosphate dehydrogenase. The results showed that without the addition of mitochondria, at a concentration of 200 μM, cyanamide had no direct effect on yeast aldehyde dehydrogenase activity. However, under these same conditions with the addition of mitochondria, complete inactivation of aldehyde dehydrogenase occurred. Similar results were obtained using rabbit skeletal muscle glyceraldehyde-3-phosphate dehydrogenase.

DeMaster et al. (1988): The cyanamide inhibition of aldehyde dehydrogenase is dependent on the catalase, which catalyzed oxidation of cyanamide to an active metabolite. This reaction is supported by hydrogen peroxide. Cumene hydroperoxide was found to be an effective substitute for hydrogen peroxide in supporting this reaction in vitro using bovine liver catalase.

Shirota et al. (1984): Hepatic N-acetyltransferase preparations from the dog or rabbits were incubated with acetyl-S-CoA (2 μmol) and ^{14}C-cyanamide (0.4 μmol, 9.2×10^5) in vitro. Hepatic N-acetyltransferase catalyzed the transfer of the acetyl group from acetyl-S-CoA to ^{14}C-cyanamide producing N-acetyl[^{14}C]cyanamide. The rate of N-acetylation (expressed as percent of substrate acetylated/20 minutes/μg protein), estimated by monitoring the disappearance of cyanamide with time in the presence of rabbit enzyme (57.6% acetylation) from a rapid acetylator phenotype rabbit and cofactor, was nearly twice that observed with the dog enzyme (24.1% and 30.4% acetylation). Thus, the enzyme responsible for the biotransformation of cyanamide is an acetyl-S-CoA-dependent N-acetyltransferase.

Shirota et al. (1987a): The mode of action of hydrogen cyanamide as an inhibitor of aldehyde dehydrogenase was studied in vitro using Sprague-Dawley rat liver microsomes. Cyanide was formed from cyanamide by microsomes in the presence of an NADPH generating system. This reaction was time dependent and reached maximum levels within 5-10 minutes. N-hydroxycyanamide was proposed as an intermediate product of cyanamide oxidation, which was then converted to cyanide and nitroxyl. Cyanide yield is doubled by cytochrome P_{450} enzyme inductors (phenobarbital) and

reduced by P_{450} inhibitors (SKF-525A). It was suggested that the nitroxyl (not cyanide) may be the aldehyde dehydrogenase inhibitor.

Shirota et al. (1987b): Cyanide is one of the products formed in the catalase-mediated oxidation of cyanamide in vitro. An increase in cyanide formation can be achieved in vitro by adding a hydrogen peroxide source to bovine liver catalase. Cyanide formation was directly related to cyanamide and catalase concentrations and was dependent on incubation time. Ethanol, a substrate for catalase, reduces cyanide formation. At cyanamide concentrations of 1 mM, ethanol (50 mM) reduced cyanide formation by 86% at 5 minutes and by 92% after 15 minutes. When cyanamide concentrations were increased to 10 mM, cyanide formation was still inhibited by ethanol, but to a lesser extent, by 68% at 5 minutes, and by 75% at 15 minutes.

APPENDIX A

REFERENCES

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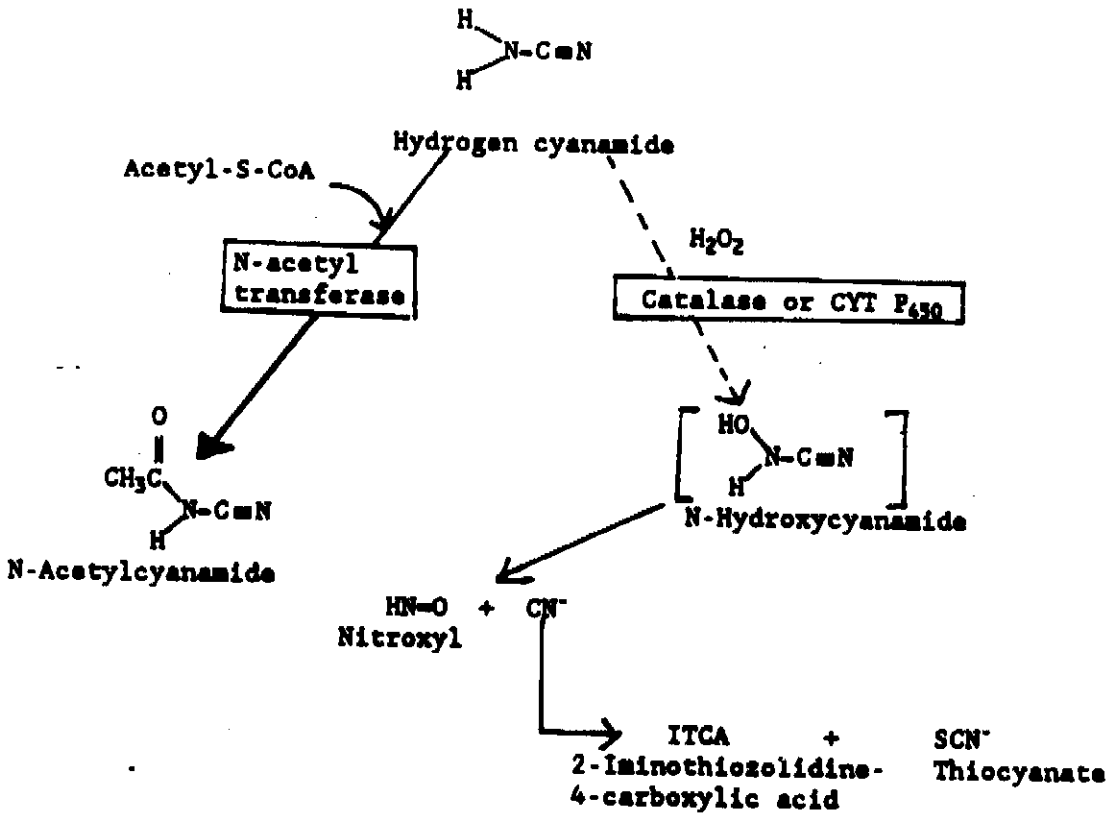


Figure 1: The Major (solid arrow) and Minor (dashed arrow) Metabolic Pathways for Hydrogen Cyanamide

(Source: Shirota and Nagasawa 1988)

FINAL

010591

DATA EVALUATION REPORT

Hydrogen Cyanamide

Study Type: A Dermal Absorption Study in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer Jessica M Kidwell Date 8/13/93
Jessica Kidwell, M.S.

Independent Reviewer William L. McLellan Date 8/13/93
William McLellan, Ph.D.

QA/QC Manager Sharon A. Segal Date 8/13/93
Sharon Segal, Ph.D.

Contract Number: 68D10075
Work Assignment Number: 2-78
Clement Number: 241
Project Officer: Caroline Gordon

EPA Reviewer: Paul Chin, Ph.D. Signature: Paul Chin
Review Section II, Toxicology Branch I, HED Date: 8/16/93

EPA Section Head: Marion Copley, D.V.M. Signature: Marion P. Copley
Review Section IV, Toxicology Branch I, HED Date: 8/19/93

DATA EVALUATION REPORT 010591

STUDY TYPE: Dermal absorption study (85-2)

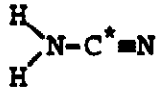
EPA IDENTIFICATION NUMBERS:

PC Code: 014002
Tox. Chem. No.: ~~266B~~ 140
MRID Number: 415040-04

TEST MATERIAL: Hydrogen cyanamide

SYNONYM: Dormex®

CHEMICAL STRUCTURE:



(*denotes position of radiolabel)

STUDY NUMBER: HLA 6265-100

SPONSOR: SKW TROSTBERG AG, D-8823 Trostberg, Federal Republic of Germany

TESTING FACILITY: Hazleton Laboratories America, Inc., Madison, WI

TITLE OF REPORT: Dermal Absorption of ^{14}C-Hydrogen Cyanamide in Male Rats

AUTHOR: Leon W. LeVan, Ph.D.

REGULATORY COMPLIANCE: A Good Laboratory Practice Compliance Statement and a Quality Assurance Statement, both signed and dated 12/08/89, were included.

STUDY COMPLETION: December 8, 1989

CONCLUSIONS: A single dermal dose of 0.1, 1, or 10 mg (8, 80, or 800 µg/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. The data can be utilized for risk assessment purposes as a worst case scenario.

CLASSIFICATION: Supplementary, pending clarification of whether the material absorbed was unknown impurities (18-22%) present in the test material or pure hydrogen cyanamide. Although this study does not fulfill all the requirements

suggested in Dr. R. Zendzian's draft "Procedure for Studying Dermal Absorption," the deficiencies are not major, except for the above impurities issue, and do not affect the interpretation of the data (see Reviewers' Comments, Section C).

A. MATERIALS and METHODS

Radiolabeled Test Material

The radiolabeled test material, ^{14}C-hydrogen cyanamide, (lot number CFQ.5182) had a specific activity of 15 mCi/mmol. The radiopurity of the undiluted radiolabeled test material (determined by Amersham Corporation) was 87%-90%. It was prepared by Amersham Corporation and stored at -20°C.

Purity of Stock Solution

Radiopurity of the ^{14}C-hydrogen cyanamide stock solution was determined by thin layer chromatography (TLC) using two solvent systems. The radiopurities were determined to be 77.5% using dichloro-methane:methanol:ammonium hydroxide[6:3:1] and 82.3% using n-butanol:water:pyridine:acetic acid [4:2:0:5:1]). The study author reported that some difficulties were encountered in obtaining satisfactory chromatography of the radiolabeled test material solution, due to either decomposition of the test material on the TLC plate or to interference by the phosphoric acid present in the stock solution.

Nonradiolabeled Test Material

Nonradiolabeled hydrogen cyanamide (Dormex® 49% w/w aqueous solution) was provided by the Sponsor and was stored refrigerated. Information in Appendix B (p. 60) indicates that this formulation (49% aqueous solution) is specially stabilized.

Test Animals

Species: Rat
Strain: Crl:CD®(SD)BR
Source: Charles River Breeding Laboratories, Portage, MI
Sex: Male
Numbers: 2, 3, or 24 Rats/dose
Housing: Individual stainless steel cages (during acclimation); individual metabolism cages (during the test period)
Identification: Ear tag
Acclimation: Eight days
Age: 7-8 Weeks
Weight: 201-233 g
Feeding: Feed (Purina Certified Rodent Chow® #5002) and water provided ad libitum.
Selection: Computer-generated randomization procedure

Preparation of Dosing Suspensions/Analysis of Dosing Suspensions

The ^{14}C-hydrogen cyanamide stock solution and nonradiolabeled hydrogen cyanamide (Dormex® formulation) were diluted with deionized water and adjusted to pH 4.5 with phosphoric acid. The targeted doses were 0.1, 1, and 10 mg of hydrogen cyanamide in a volume of 100 µL for groups 4, 5, and 6, respectively. The doses were prepared as follows:

Group	^{14}C (mg)	Nonradiolabeled Test Material (mg)	Total Test Material (mg)	Total Volume (mg/mL)	Test Material Concentration (mg/mL)
4	1.0	4.0	5.0	5	1.0
5	1.0	48.8	49.8	5	10.0
6	1.0	499	500	5	100

Duplicate pre- and postdose samples of the dosing solutions for groups 4-6 were taken at the time of dose administration to determine the amount of ^{14}C applied. The specific activities of the dosing solutions were 154,000, 15,400, and 1560 dpm/µg for groups 4, 5, and 6, respectively. The mean concentration of radioactivity in the dose solutions for groups 4, 5, and 6 was determined to be 7.673, 7.706, and 7.786 x 10^7 dpm/g, respectively. The average dose of radioactivity applied to each animal was 3.45, 3.46, and 3.5 µCi for groups 4, 5, and 6, respectively.

Dosage Groups/Application of Test Material/Treatment Regimen

Twenty-four hours prior to treatment, the back and shoulders of each rat in groups 4, 5, and 6 were shaved. Care was taken to avoid damaging the skin. The shaved area of skin was washed with acetone to remove oily secretions. The dosing area was enclosed with a plastic enclosure (12.5 cm^2) glued on the middle of each animal's back with cyanoacrylate-based glue. Silicone medical adhesive was used as a seal around the outside of the enclosure. An Elizabethan collar was placed around each animal's neck. The test material was applied evenly to the test site with a glass rod. To prevent disturbance of the application site and loss of the test material, the application site was then covered with a non-occlusive cover (filter paper affixed with rubber cement). The two control animals (group 3) were treated in the same way as the treated animals, except they were dosed with the vehicle only.

The animals in the preliminary dermal irritation groups (groups 1 and 2) were treated the same as the treated and control animals except they were dosed with nonradiolabeled test material (5% or 10% Dormex in deionized water) only.

Group designations and dose levels are as follows:

Group	Number of Animals	Test Material Concentration (%w/v)	Dose of Active Ingredient (mg/animal)	Volume Applied/Animal (µL)
1[a]	3	5	5	100
2[a]	3	10	10	100
3	2	0[b]	0	100
4	24	0.1	0.1	100
5	24	1	1	100
6	24	10	10	100

[a]Animals in groups 1 and 2 were used for preliminary examination of dermal irritation and dosed with unlabeled test material only.
[b]Vehicle control

Observations

All animals were observed daily for moribundity and mortality.

Sample Collections

No samples were collected from animals in the preliminary dermal irritation study.

At 0.5, 1, 2, 4, 10, and 24 hours postdosing, four animals per dose (groups 3-6) were euthanized. Single samples of urine, feces, and blood were collected from the time of dosing to the time of euthanization. Any residual urine present in the bladder at sacrifice was collected and added to the sample. Cage washings were also collected after each sacrifice. The skin at the application site (with cover in place) and carcass were collected for analysis.

The urine, glass rod rinses, protective appliance rinses, cage wash, and cage wipe were analyzed in duplicate to determine ^{14}C using a liquid scintillation counting (LSC) system. Duplicate aliquots of feces and carcass (both weighed and homogenized) and blood were combusted in a sample oxidizer. Measurement by liquid scintillation techniques was used for all analyses of ^{14}C.

Skin Wash Procedure

The skin wash procedure was performed immediately after sacrifice. After excision of the application site skin, the skin was washed repeatedly (minimum nine times each) with 5% soap (Ivory® liquid) followed by deionized water. The equipment used in the washing procedure was rinsed with methanol, and the rinse was combined with the skin wash liquid. The total weight of the skin was taken, and duplicate aliquots were counted directly using LSC.

Calculation of Quantity Absorbed

The amount of ^{14}C-hydrogen cyanamide was determined using two procedures, direct (used by the study author) and indirect (calculated by the reviewers). The direct procedure calculated the amount of

^{14}C-hydrogen cyanamide absorbed by summing the percentages of dose recovered in the excreta (urine, feces), cage wash, cage wipe, and the carcass for each rat. The indirect procedure calculated the amount of unabsorbed ^{14}C-hydrogen cyanamide by summing the amount of ^{14}C washed from the protective appliance, the amount washed from the skin, and the amount associated with the skin. The total amount of ^{14}C-hydrogen cyanamide absorbed using indirect calculation, therefore, was equal to the applied dose (100%) minus the calculated unabsorbed ^{14}C-hydrogen cyanamide.

B. RESULTS/CONCLUSIONS

In the preliminary dermal irritation study, there was no sign of dermal irritation following exposure to either 5% or 10% Dormex® for 24 hours. In the test group, no clinical signs were noted that were attributable to the test material.

The disposition of ^{14}C-hydrogen cyanamide equivalents and the calculated absorbed doses are presented in Table 1.

Direct Procedure

A single dermal dose of 0.1, 1.0, or 10.0 mg/animal ^{14}C-hydrogen cyanamide administered to rats was rapidly absorbed, distributed and eliminated. In general, as the dose level and the duration of exposure increased, the percentage of the dose absorbed increased. Using the direct procedure to calculate absorption through the skin, the average amounts of ^{14}C-hydrogen cyanamide absorbed within 0.5 hour were 0.07%, 0.16%, and 0.12% of the applied dose for the low-, mid-, and high-dose groups, respectively. At 1 hour postdosing, the average amounts of ^{14}C-hydrogen cyanamide were 0.19%, 0.29%, and 0.66% of the applied dose for the low-, mid-, and high-dose groups, respectively. The average amounts absorbed increased with time such that by 24 hours the amounts absorbed were 1.79%, 2.84%, and 11.1% of the applied dose for the low-, mid-, and high-dose groups, respectively. The study author reported that these values correspond to total absorbed doses of 1.79, 28.4, and 1100 µg equivalents over the 24-hour exposure period for doses of 0.1, 1, and 10 mg ^{14}C-hydrogen cyanamide, respectively.

The absorption data indicated that ^{14}C-hydrogen cyanamide penetrated the skin within the first 0.5 hour of exposure as indicated by mean blood radioactivity concentrations of <0.001, 0.006, and 0.029 µg/g of blood for the low-, mid-, and high-dose groups, respectively. In the low-dose group, mean blood radioactivity increased with time, ranging from <0.001 µg/g (not detectable) at 2 hours to 0.002 µg/g (maximum level) at 24 hours. In the mid- and high-dose groups, mean blood radioactivity levels increased with dose and time up to 10 hours and decreased between 10 and 24 hours. In the mid-dose group, mean blood radioactivity ranged from 0.006 µg/g at 0.5 hour to 0.037 µg/g (maximum level) at 10 hours postdosing and decreased to 0.030 µg/g at 24 hours postdosing. In the high-dose group, mean blood radioactivity ranged from 0.029 µg/g at 0.5 hour to 1.04 µg/g (maximum level) at 10 hours postdosing and decreased to 0.780 µg/g at 24 hours postdosing.

The decrease in blood radioactivity between 10 and 24 hours was not statistically significant ($p<0.05$ using Student's t-test). The maximum blood radioactivity concentrations in the rats at each dose level were proportionately higher than the 10-fold difference in dose levels.

Urinary excretion of radioactivity increased with time and dose. Urinary radioactivity was detectable in all dose groups at 10 and 24 hours postexposure. Maximum mean recoveries of radioactivity in urine occurred at 24 hours postdosing and were 0.93%, 1.69%, and 7.65% for the low-, mid-, and high-dose groups, respectively. Fecal excretion of radioactivity was very low (≤0.2% of the applied dose) for all dose groups and for all durations. Maximum mean recoveries at 24 hours were 0.04%, 0.05%, and 0.20% for the low-, mid-, and high-dose groups, respectively.

^{14}C was rapidly distributed to the carcass and was detected within the first 0.5 hour at 0.07%, 0.16%, and 0.12% for the low-, mid-, and high-dose groups, respectively. Carcass radioactivity peaked at 24 hours in the low-dose group (0.65% of the dose), and at 10 hours in the mid- (1.07% of the dose) and high-dose (2.35% of the dose) groups. In the mid- and high-dose groups, radioactivity decreased to 0.89% and 2.05%, respectively, at 24 hours postdosing.

Total average recoveries of applied radioactivity ranged from 94.20% to 104.91% in the low-dose group, 93% to 103.11% in the mid-dose group, and 86.27% to 98.23% in the high-dose group.

Indirect Procedure

Using the indirect procedure, the average amounts of ^{14}C-hydrogen cyanamide absorbed after 24 hours were 7.6%, 9.87%, and 21.67% of the dose for the low-, mid-, and high-dose groups, respectively. The direct procedure for calculation of absorption was more realistic, as the indirect calculation produced some negative numbers, and absorption values were considerably higher than those calculated by the direct procedure; therefore, emphasis should be placed on the direct absorption values.

The majority of the unabsorbed ^{14}C-hydrogen cyanamide was associated with the skin washes. For the low-, mid-, and high-dose groups, 96.9%, 97.5%, and 89.6%, respectively, of the applied dose were associated with skin washes at 0.5 hour postexposure. The amount of ^{14}C associated with skin washes decreased after 24 hours to 69%, 67.7%, and 52.9%, for the low-, mid-, and high-dose groups, respectively. The amounts of ^{14}C associated with the skin site at 0.5 hour postexposure were 6.57%, 4.41%, and 7.47% of the applied dose for the low-, mid-, and high-dose groups, respectively. At 24 hours postexposure they were 17%, 13.5%, and 11.1% of the applied dose for the low-, mid-, and high-dose groups, respectively. The amount of ^{14}C associated with the protective appliance increased with time. In the low-, mid-, and high-dose groups, approximately 1% of the applied dose was recovered at 0.5 hour post-exposure. By 24 hours, 6.4%, 8.93%, and 14.33% of the applied dose were recovered in the low-, mid-, and high-dose groups.

C. REVIEWERS' COMMENTS

010591

This study was classified as supplementary because of the following reason: It is not clear whether the radiolabeled material absorbed was pure hydrogen cyanamide or unidentified impurities (up to 22%) present in the test article.

Although this study did not fulfill all the requirements suggested in Dr. R. Zendzian's[1] draft "Procedure for Studying Dermal Absorption," the deficiencies, except for the above impurities issue, were not major and did not affect the interpretation of the data. The following deficiencies were noted: (1) The skin wash was taken after the animals were sacrificed. The exact time of the collection following sacrifice was not stated. The general procedures state that the skin wash should be collected before the animals are sacrificed, as up to 3-fold differences have been observed in the ability of skin on the live animal and skin on the dead animal to bind test compounds; (2) According to Dr. R. Zendzian's draft procedure, "The highest useful dose is in the order of 10 mg/rat with descending doses of 1, 0.1, and 0.01 mg/rat. If less than four doses are used it is preferred that the lower dose range (1, 0.1, and 0.01 mg/rat) be used." In this study, the highest three doses (10, 1, and 0.1 mg/rat) were used instead of the recommended dose range, as indicated above.

[1]R.P. Zendzian, "Procedure for Studying Dermal Absorption", Office of Pesticides Program, EPA. June 28, 1990. Fourth Editon, Revised September 18, 1987, including California Modifications October 9, 1985.

TABLE 1: The Average Percent Disposition of ^{14}C Equivalents Following a Single Dermal Exposure to Doses of ^{14}C-Hydrogen Cyanamide in Male Rats[a,b]

Time Euthenized (hours)	Protective Appliance Rinses (%)	Skin Washes (%)	Associated With Skin at Site (%)	Blood (µg/g)[c]	Urine (%)	Feces (%)	Cage Wash (%)	Cage Wipe (%)	Carcass (%)	Average Recovery of Applied ^{14}C-HC (%)	Average ^{14}C-HC Absorbed (Direct Procedure) (%)	Average ^{14}C-HC Absorbed (Indirect Procedure) (%)
					0.1 mg/animal (8 µg/cm^2)							
0.5	1.33	96.9	6.57	<0.001	ND	ND	ND	0.01	0.07	104.91	0.07	-4.80
1	1.23	89.3	6.80	<0.001	0.02	ND	ND	0.01	0.17	97.55	0.19	2.67
2	2.38	88.2	7.48	ND	0.05	ND	ND	0.01	0.08	98.15	0.13	1.94
4	2.62	88.9	6.48	ND	0.17	NS	ND	0.01	0.10	98.28	0.27	2
10	4.34	86.0	7.03	0.001	0.70	ND	ND	0.01	0.50	98.58	1.20	2.63
24	6.40	69.0	17.00	0.002	0.93	0.04	0.16	0.03	0.65	94.20	1.79	7.6
					1.0 mg/animal (80 µg/cm^2)							
0.5	1.10	97.5	4.41	0.006	ND	ND	ND	<0.01	0.16	103.11	0.16	-3.01
1	2.03	92.3	4.03	0.008	ND	ND	ND	<0.01	0.29	98.68	0.29	1.64
2	3.10	85.1	8.67	0.022	<0.01	ND	0.03	0.02	0.65	97.56	0.69	3.13
4	4.72	86.9	5.99	0.011	ND	NS	ND	0.01	0.53	98.10	0.53	2.39
10	7.9	77.6	6.81	0.037	1.45	0.02	0.07	0.04	1.07	94.97	2.64	7.69
24	8.93	67.7	13.5	0.030	1.69	0.05	0.19	0.03	0.89	93.00	2.84	9.87
					10.0 mg/animal (800 µg/cm^2)							
0.5	1.09	89.6	7.47	0.029	0.01	ND	ND	ND	0.12	98.23	0.12	1.84
1	2.45	81.9	11.7	0.291	ND	ND	0.01	0.02	0.65	96.73	0.66	3.93
2	4.89	81.5	9.46	0.384	ND	NS	ND	<0.01	0.91	96.74	0.91	4.15
4	7.57	71.5	5.33	0.639	0.22	ND	ND	<0.01	1.63	86.27	1.85	15.60
10	12.51	65.8	6.81	1.04	4.38	0.06	0.60	0.15	2.35	92.67	7.53	14.88
24	14.33	52.9	11.1	0.780	7.65	0.20	0.93	0.22	2.05	89.41	11.1	21.67

[a]Each value represents the mean of four rats.
[b]Values are expressed as percentage of applied dose.
[c]The µg/g values refer to the mean concentration of ^{14}C-hydrogen cyanamide equivalents in the blood.

Abbreviations used: HC = hydrogen cyanamide; ND = not detected; NS = no sample

Source: Tables 5-14; pp. 31 and 33-40

Reviewed by: William B. Greear, M.P.H. William B. Greear 9/21/93
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 9/21/93
Review Section IV, Toxicology Branch I (H7509C)

010591

DATA EVALUATION REPORT
(Supplement 1 to Study No. 3319-121; MRID # 412888-02
(TOX DOC # 008150)

STUDY TYPE: Guideline Series 83-1
Chronic Toxicity in Dogs

Tox. Chem. No.: 140
PC No.: 014002
MRID No.: 412888-02

TEST MATERIAL: Aqueous Hydrogen Cyanamide 50% w/w, clear colorless material
Lot No. 07/07/87 (=530 g/l)

SYNONYMS: Carbimide, Carbodiimide, Amidocyanogen, Cyanogenamide, SKW 83010, CAS 420-04-2

STUDY NUMBER: 2319-121

SPONSOR: SKW Trostberg AG
D-8223 Trostberg, FRG
(Siemer & Associates, Inc.)

TESTING FACILITY: Hazleton Laboratories, Inc.
Vienna, VA 22180

TITLE OF REPORT: Chronic Toxicity Study in Dogs With Aqueous Hydrogen Cyanamide

AUTHOR: M.R. Osheroff

REPORT ISSUED: May 10, 1989

CONCLUSIONS:

Aqueous Hydrogen Cyanamide (50% w/w, 53% w/v a.i.) was administered by gavage to groups of 4 male and 4 female dogs at dose levels of 0.4, 2.0 or 10.0 mg/kg/day (equivalent to 0.2, 1.0 or 5.0 mg/kg/day a.i.)

[For the first 2 weeks the dogs were administered 0.2, 1.0 or 5.0 mg/kg/day of the 50% technical.]

NOEL = 0.2 mg/kg/day a.i.
LEL = 1.0 mg/kg/day a.i. (based on decreases in MCV and MCH in males and females, and an increase in pale areas of the spleen in males)

In addition, at 5.0 mg/kg/day a.i. there were increases in males and females with rough haircoat and desquamation of the skin, tremors, salivation, decreases in body weight gain, leukocyte counts (males), albumin, calcium (females), phosphorous (males) and T_4 (males) and an increase in the relative weight of the thyroid-

parathyroid (females), a possible decrease in sperm activity (males).

010591

Classification: Core-Guideline

Study Acceptability: The study satisfies the requirement for a Guideline Series 83-1 Chronic Toxicity in Dogs. Study.

This supplement does not change the conclusions from the previous DER, only clarifies them.

010591

Reviewed by: William B. Greear, M.P.H. William B. Greear 9/23/93
Review Section IV, Toxicology Branch I (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion Copley 9/21/93
Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT
(SUPPLEMENT 1 TO MRID NO. 415665-02)
(TOX DOC. # 008422)

Study Type: Guideline Series 83-2b
Oncogenicity
Study in Mice

Tox. Chem. No.:140
PC No.:014002
MRID No.:421784-05

Test Material: Hydrogen Cyanamide

Synonyms: Carbimide, Amidocyanogen, Cyanamide, Cyanogenamide, carbodiimide, carbamonitrile, Cyanogen nitride

Study Number: 6001-556/3

Sponsor: SKW Trostberg AG., Trostberg, Germany

Testing Facility: Hazleton UK, North Yorkshire, England

Title of Report: Hydrogen Cyanamide, Up to 104 Week Oral (Drinking Water) Carcinogenicity Study

Author: M.J. Goodyer

Report Issued: October 1991

CONCLUSIONS:

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, 13.7, and 36.8 mg/kg/day a.i.; for females: 0, 6.6, 16.9, and 49.0 mg/kg/day a.i.).

NOEL = 70 ppm (6.9 mg/kg/day a.i.)
LEL = 200 ppm 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 there was decreased body weight gain (significant); in females 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.

Classification: Core-Minimum. This study satisfies the requirement for a guideline series 83-1b oncogenicity study in mice.

RESULTS AND DISCUSSION:

This study (initially reviewed in HED Doc. # 008422 dated 6/24/91) was classified as core-supplementary until the following data/information was provided:

1 Data to verify the stability of dosing solutions.

2 Data for clinical signs and palpable masses for individual animals.

3 An explanation why note entries on the histopathology sheets for "borderline with granulosa-theca tumors" were not included in the tumor analysis.

4 Detailed historical control data on the incidence of granulosa-theca tumors in this strain of mouse used at Hazleton, UK.

The Registrant submitted responses to the above deficiencies.

1 (MRID # 421784-05) The report states that the actual range of concentrations seen during the study was 91-117 % (of nominal). Most of the results were noted as being 100 % ± 10 %. Additional details were presented on pages 111 and 112 of the report.

2 Tables were presented for clinical signs, tissue mass incidence tissues examined, missing tissues and autolytic tissues.

3 (MRID # 421784-05) Based on the definition provided by the study pathologist (attached - taken from pages 8-9 of study report) for use of the term "granulosa-theca tumor", Dr. Brenneke (consultant for HED, EPA) determined that it is appropriate to include the following lesion listings in the ovarian tumor tables in combination with "granulosa-theca tumors".

- borderline with granulose-theca tumor
- borderline with luteinized thecoma
- borderline with granulosa-theca-luteal tumor
- borderline with luteoma
- borderline with thecal cell tumor
- borderline with microscopic luteoma

Therefore, the total tumor count for this type of tumor is: 3, 1, 7 and 13 for the control, low,mid and high dose

groups, respectively. This data will be evaluated by the HED statistics group for the Carcinogenicity Peer Review.

4 (MRID # 421784-05) The historical control data submitted by the Registrant are attached.

The deficiencies noted in the original DER have been satisfactorily responded to. This study is therefore upgraded to core-minimum and satisfies the requirement for an oncogenicity study in mice.

The HED Carcinogenicity Peer Review Committee considers these tumors to be related to treatment (Doc. 9/15/93).

HYDROGEN CYANAMIDE, UP TO 104 WEEK ORAL (DRINKING WATER) CARCINOGENICITY STUDY IN THE MOUSE (HUK STUDY NO 6001-556/3): SUPPLEMENTARY COMMENTS ON GRANULOSA-THECA TUMOURS

Introduction

The above study was conducted by Hazleton UK (North Yorkshire, England), on behalf of SKW Trostberg AG (Trostberg, Germany) as sponsor. The final report was issued on May 3, 1990.

Upon request of the sponsor Hazleton UK was asked to supply additional data for clarification of the results in the report. As far as the pathology section is concerned Hazleton UK should specify the criteria of assessment and classification which were used to evaluate the ovarian findings from the above study.

Regarding this question Hazleton UK has prepared the following explanatory note to the best of our knowledge. It is based upon more than 20 years experience in pathology work as well as on generally recognised principles of tumour diagnosis and the available historical background incidence data on granulosa-theca tumours in Crl:CD-1(ICR)BR mice.

Background comments on Ovarian Neoplasms

Ovarian neoplasms are uncommon in CD-1 mice and little is known about mechanisms. In general, a range of 0 to 3 is usually found in the standard carcinogenicity studies containing 50-51 females per control group (Table 3). Because of this low incidence it was the general practice in Hazleton UK to pool tumours or the sex-cord stromal elements under the general diagnostic term "Granulosa-theca tumour". This practice was adopted to increase the sensitivity of statistical analysis of low incidence tumours and was also applied to other low incidence neoplasms such as gliomas in the brain.

Study Data

Eight benign granulosa-theca tumours were reported in high dose females. The tumours were generally microscopic entities. The conventional subclassifications of these tumours include granulosa cell tumour, luteoma, thecoma and tubulo-stromal adenoma. Most of the cases in this study were

of the luteoma form of granulosa-theca tumour as indicated in the free text comments associated with the reported diagnoses. Diagnosis of these tumours is confounded by cysts, stromal/luteal hyperplasias and various other conditions affecting the aged ovary, and the main criterion for diagnosis was a nodular proliferation distorting or compressing the surrounding ovarian tissue. Distinction between these nodules of luteal proliferation and more extreme forms of stromal and luteal hyperplasia are not clear-cut.

In borderline cases, the pathologist classified the finding as hyperplasia or neoplasia based on his judgement and experience. However, where such borderline judgements were made, a free text note was made to this effect so that reviewing pathologists could identify cases where there was some degree of uncertainty. There was also an element of uncertainty in the diagnosis of a necrotic tumour in one control, but on balance, the diagnosis was judged to be granulosa-theca tumour. This uncertainty in some diagnoses is unavoidable in a subjective discipline such as pathology, but decisions have to be made for the purpose of statistical analysis.

The judgement of the pathologist on borderline cases led to a relatively conservative statistical analysis compared with one that could have been based, perhaps wrongly, on the inclusion of borderline tumours and exclusion of the necrotic control. However, the difference would have been quantitative rather than qualitative and would only reinforce, not alter the basic conclusion that the incidence of tumours in high dose females was greater than in the controls and historical control data.

Conclusion

Ovarian tumours are generally reported as rare in CD mice. The granulosa-theca tumours found in this study were generally microscopic and usually of the luteoma sub-type. Especially in the high dose females they occurred at a higher incidence than that in the study control group and that usually found in other groups of control females. Some luteal hyperplasias and microscopic tumours are similar morphologically and borderline cases do occur. However, changing the allocation of the equivocal control case and borderline high dose females does not alter the basic qualitative conclusion that the incidence of tumours in high dose animals exceeded that in the controls.

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TABLE 3

HAZLETON UK HISTORICAL CONTROL TUMOUR INCIDENCE (%)
OVARY: GRANULOSA-THECA TUMOURS IN Crl: CD-1(ICR)BR MICE

CONTROL GROUP	TERM (YEAR)	DURATION (WEEKS)	NO. ANIMALS PER GROUP	INCIDENCE (%)	#
1	1981	104	60	5	3
2	1981	104	60	0	0
3	1981	80	50	0	0
4	1981	80	50	4	2
5	1979	100	70	0	0
6	1980	104	90	1	1
7	1982	80	100	0	0
8	1981	80	51	6	3
9	1982	91	51	0	0
10	1982	80	51	6	3
11	1982	80	51	0	0
12	1983	104	49	4	2
13	1984	91	51	0	0
14	1984	91	51	0	0
15	1984	83	51	0	
16	1985	104	51	0	
17	1985	104	51	0	
18	1985	88	51	0	
19	1985	88	51	2	1
20	1986	80	51	0	
21	1987	104	51	6	3
22	1987	104	51	0	
23	1987	104	51	0	
24	1986	104	62	2	1
25	1987	104	51	2	1
26	1987	104	51	0	
27	1988	78	102	1	
28	1988	83	102	1	
29	1988	80	51	0	
30	1989	104	51	0	
31	1989	104	50	2	1
32	1989	94	51	0	
33	1990	80	51	2	1
34	1990	80	51	0	

014002

Reviewed by: William B. Greear, M.P.H. William B. Greear 9/23/93
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Review Section IV, Toxicology Branch I (H7509C)

DATA EVALUATION REPORT
(Supplement 3 to TOX Doc # 008162)

Study Type: Oncogenicity - Mouse 83-2b
Tox. Chem. No.: 140A
PC Code: 014001
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, Cyanamid Granular, Cyanamid Special Grade

Study Number: (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: The Carcinogenicity Peer Review Committee (document dated 9/15/93) concluded that:

"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."

B6C3F1 mice were treated with 0, 500 or 2000 ppm (approximately 0, 37 or 150 mg/kg/day a.i.) in the diet.

NOEL = 500 ppm
LEL = 2000 ppm based on increased mortality (males).

(there was a slight decrease in body weight at both doses but it could not be adequately evaluated (graph only).

Carcinogenic Potential: treatment related increase in hemangiosarcoma (males).

Core Grade: Minimum. This study satisfied the guideline requirement for a 83-2b study for Calcium Cyanamide or hydrogen Cyanamide.

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 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 3. 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 3. Calcium Cyanamide - NTP Study

Male Hemangiosarcoma Rates in B6C3F1 Mice*

	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 or Exact trend test).

* Abstracted from the Memorandum from W. Greear dated January 20, 1987, HED DOC. No. 008661 (DER for the NTP Mouse Oncogenicity Study).

The historical control incidence of hemangiosarcomas in male B6C3F1 mice at the testing laboratory was 13/327 (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 an artificial 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 these tumors. Hemangiosarcoma is a malignant tumor, it may have been significantly elevated above control by pairwise comparison if an adequate number of control animals had been included, and the rate of tumor formation at the HDT (20%) is very high, according to Dr. Lucas Brennecke (Pathology Associates Inc.). For these reasons, the CPRC considers hemangiosarcoma a compound-related tumor in male mice at the HDT.

The incidence of lymphoma/leukemia in female control animals was below the lower end of the range of 4/5 historical control studies. 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. It was difficult for the CPRC to arrive at conclusions regarding the biological significance of lymphoma/leukemia in female mice with the limited histopathological data at hand. Therefore, the CPRC agreed to accept the conclusions of the NCI regarding this tumor type, and did not consider lymphoma/leukemia in female mice to be compound-related.

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Final
MC
8/11/93

DATA EVALUATION REPORT

(Addendum to Study No. (NIH) 79-1719; Doc# 005681, 005682)

Calcium Cyanamide

Study Title: Stability of Calcium Cyanamide, Technical Grade, in Animal Diet with Regards to the NCI Bioassay 83-2a, b
(Correction of dose levels in Rat DER)

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

Principal Reviewer	Jessica Kidwell Jessica Kidwell, M.S.	Date 6/14/93
Independent Reviewer	William McLellan William McLellan, Ph.D.	Date 6/14/93
QA/QC Manager	Sharon Segal Sharon Segal, Ph.D.	Date 6/14/93

Contract Number: 68D10075
Work Assignment Number: 2-78
Clement Number: 242
Project Officer: Caroline Gordon

EPA Reviewer: William B Dr... er, M.P.H. Signature: William B. Dr...
Review Section IV, Toxicology Branch I, HED Date: 8/16/93

EPA Section Head: MARION P. Copley, D.V.M. Signature: Marion Copley
Review Section IV, Toxicology Branch I, HED Date: 8/16/93

DATA EVALUATION REPORT

TEST MATERIAL: Calcium cyanamide
PC Code: 019001
MRID Number: 415040-02
Tox Chem No 14C
PERFORMING LABORATORY: SKW Trostberg AG, Analytical Department, Dr. Albert-Frank-Str. 32, D-8223 Trostberg, Federal Republic of Germany

TITLE OF REPORT: Stability of Calcium Cyanamide, Technical Grade, in Animal Diet

AUTHOR: Dr. Ulrich Rust

REGULATORY COMPLIANCE: A Good Laboratory Practice Compliance Statement and a Quality Assurance Statement were not included.

STUDY COMPLETION: May 11, 1987

CONCLUSIONS: The stability of calcium cyanamide in animal diet used for an NCI cancer study was determined by SKW Trotsberg. Hydrogen cyanamide is regarded as the active ingredient of calcium cyanamide because calcium cyanamide is rapidly converted to hydrogen cyanamide. The test diet, Wayne Sterilizable Lab Meal (400 g) containing 4% fat, was spiked with 41.35 mg calcium cyanamide containing 27.3% hydrogen cyanamide. The concentration of hydrogen cyanamide in the diet was 28.2 ppm (determined by calculation based on amount added to diet). The spiked sample was homogenized and then stored in a refrigerator at 7°C. The hydrogen cyanamide content was analyzed 7, 12, and 17 days after preparation using high performance liquid chromatography (HPLC) with a variable wavelength detector following derivatisation with 1,2-naphthoquinone-4-sulfonate. Recovery analysis was not performed on day 0. Recoveries of hydrogen cyanamide 7, 12, and 17 days postpreparation were 69%, 79%, and 79%, respectively (Table 1). These recovered percentage values were determined using the calculated amount, not the analyzed amount, for day 0. Although a recovery experiment was not performed on day 0, the recoveries for days 7, 12, and 17 indicate that the stability of calcium cyanamide in the diet preparations used in the NCI study is satisfactory.

CLASSIFICATION: Supplementary (This addendum)
Main report is already Minimum

Note: the original DER for the rat cancer study had incorrect dose Levels noted for the female. The male doses were 0, 100, 200, while the females were 0, 100, 400 ppm.

Table 1

	Date of analyses	Sample weight (g)	Peak Height (mm)	Amount of cyanamide (ppm)	Recovery (%)
Standard	11/28/86	--	144	--	--
Test material	11/28/86	50 g	72	19.4	69
Standard	12/03/86	--	147	--	--
Test material	12/03/86	50 g	85	22.4	79
Standard	12/08/86	--	123	--	--
Test material	12/08/86	50 g	73	22.3	79

Primary Reviewer: William B. Greear, M.P.H. William B. Greear 9/23/93
Section IV, Tox. Branch I, HED (H7509C)
Secondary Reviewer: Marion P. Copley, D.V.M. Marion P. Copley 9/21/93
Section IV, Tox. Branch I, HED(H7509C)

DATA EVALUATION REPORT

(Supplement 1 to MRID # 412888-05)
(TOX Doc #008150)

Study Type: Developmental Toxicity Study - Rabbits (83-3)
TOX Chem No.: 140
PC No.: 014002

MRID No.: 421784-06

Test Material: Hydrogen Cyanamide (49 % w/w)

Synonyms: Dormex, Carbamide, Amidocyanogen, Carbamonitrile, Cyanogen Nitride, Cyanogenamide

Study No.: V 91.391 (main study B 84-0171)

Sponsor: SKW Trostberg AG

Testing Facility: TNO-CIVO Toxicology and Nutrition Institute
Department of Biological Toxicology
Utrechtseweg 48, 3704 HE Zeist, Netherlands

Title of Report: Preparation of Various Aqueous Cyanamide Solutions and Analysis of these Solutions for Actual Content, Homogeneity and Stability, (Main study: Oral Embryotoxicity/ Teratogenicity Study with an Aqueous Cyanamide Solution (Content 49%) in New Zealand White Rabbits (Final Report-Second Revised Version))

Authors: I.J.Vuik (main study: H.B.W.M. Koeter and M.W. vanMarwijk)

Report Issued: 9/5/91 (main study: May 1989)

Conclusions: NOEL (maternal toxicity) = 12 mg/kg (6 mg/kg a.i.)
LEL (maternal toxicity) = 36 mg/kg (18 mg/kg a.i.)
(based on decreased body weight gain)

NOEL (developmental) = 12 mg/kg (6 mg/kg a.i.)
LEL (developmental) = 36 mg/kg (18 mg/kg/a.i.)
(based on disintegration of liver structure and slight decrease in fetal size and weight)

Core Classification: Core Minimum (data on the analytical concentration, stability and homogenicity data on the dosing solutions were provided in MRID #421784-

06).

Study Acceptability: The study satisfies the requirement of a Guideline Series 83-3 Developmental Toxicity Study.

Discussion:

Study deficiencies: These were adequately addressed in the supplement.

Issues: There are 2 independent DERs in the HED files. The first (TOX DOC # 005681) was prepared from the first submission of this study (dated Nov., 1984). The second DER (TOX DOC # 008150) was prepared from a second revised version, (May, 1989). The RfD committee developmental specialists agreed with the conclusions of the second DER. This one supersedes/replaces the first. The separate reference to the first study in the one-liners will be deleted.

The following additional supporting data tables from the study report are attached to this supplement.

1) Reproductive parameters, taken from tables 3, 7, 8, pages 22, 27- 30.
2) Maternal body weight values, taken from table 4, page 23.
3) Select fetal data, taken from table 11, page 32)

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TABLE 3 - MATERNAL PERFORMANCE

	mg cyanamide solution/kg/day			
	0	4	12	36
Number of animals inseminated	25	24	24	24[1]
Number of inseminated animals showing evidence of ovulation	25	24	24	24
Number of pregnant animals	23	20	23	20
Fertility index[2]	92.0	83.3	95.8	83.3
Preterm deliveries	1[3]	0	2	0
Number of animals that died during the experiment	1[3]	0	0	1
Number of animals with total litter loss	0	0	0	1
Number of animals with live foetuses	22	20	21	18
Gestation index[4]	95.6	100.0	91.3	90.0

1) Animal 849 showed evidence of pre-existing pregnancy and was removed from the experiment

2) Fertility index = $\frac{\text{number of pregnant rabbits} \times 100}{\text{number of inseminated rabbits, showing evidence of ovulation}}$

3) Same animal

4) Gestation index = $\frac{\text{number of rabbits with live foetuses} \times 100}{\text{number of pregnant rabbits}}$

CIVO/TNO
STUDY NO. 62? ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN NZW-RABBITS ID.NO = 5733

TABLE: 4 MEAN MATERNAL BODY WEIGHTS DURING PREGNANCY

F E M A L E S

		DAY 0 BODY WGT (GRAMS)	DAY 6 BODY WGT (GRAMS)	DAY 19 BODY WGT (GRAMS)	DAY 29 BODY WGT (GRAMS)	DAY 29 ADJ.BW. (GRAMS)
CONTROL	MEAN	3741.3	3846.1	3954.8	4012.3	3530.6
	SEM	88.6	90.0	96.6	104.4	94.0
	N	23	23	23	22	22
4 MG/KG/DAY	MEAN	3821.0	3943.0	4062.5	4136.5	3700.3
	SEM	74.1	85.2	90.2	89.6	86.0
	N	20	20	20	20	20
12 MG/KG/DAY	MEAN	3736.7	3790.4	3876.1	3939.0	3509.6
	SEM	69.4	73.8	79.0	77.5	71.2
	N	23	23	23	21	21
36 MG/KG/DAY	MEAN	3749.3	3839.8	3829.5	3871.1	3512.0
	SEM	68.8	68.2	85.1	86.4	80.0
	N	20	20	20	19	18

STATISTICS: COVAR + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

BODY WGT = BODY WEIGHT
ADJ.BW. = BODYWEIGHT AT SACRIFICE -GRAVID UTERUS WEIGHT

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Table 4

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ID.NO - 5910

STUDY NO. 629 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN NZW-RABBITS

TABLE:7 CONTINUED 1

FEMALES

		ER LEFT	ER RIGHT	ER LTTR	LR LEFT	LR RIGHT	LR LTTR	DF LEFT	DF RIGHT
CONTROL	MEAN	0.2	0.1	0.35	0.1	0.1	0.17	0.1	0.1
	SEM	0.1	0.1	0.14	0.1	0.1	0.10	0.1	0.1
	N	23	23	23	23	23	23	22	22
4 MG/KG/DAY	MEAN	0.2	0.2	0.45	0.2	0.2	0.30	0.2	0.2
	SEM	0.1	0.1	0.22	0.1	0.1	0.13	0.1	0.1
	N	20	20	20	20	20	20	20	20
12 MG/KG/DAY	MEAN	0.0	0.3	0.32	0.1	0.1	0.23	0.1	0.1
	SEM	0.0	0.1	0.12	0.1	0.1	0.23	0.1	0.1
	N	22	22	22	22	22	22	21	21
36 MG/KG/DAY	MEAN	0.4	0.4	0.85	0.1	0.1	0.15	0.3	0.3
	SEM	0.2	0.3	0.43	0.1	0.1	0.08	0.2	0.2
	N	20	20	20	20	20	20	19	19

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

ER LEFT = NUMBER OF EARLY RESORPTIONS LEFT HORN
ER RIGHT = NUMBER OF EARLY RESORPTIONS RIGHT HORN
ER LTTR = NUMBER OF EARLY RESORPTIONS PER LITTER
LR LEFT = NUMBER OF LATE RESORPTIONS LEFT HORN
LR RIGHT = NUMBER OF LATE RESORPTIONS RIGHT HORN
LR LTTR = NUMBER OF LATE RESORPTIONS PER LITTER
DF LEFT = NUMBER OF DEAD FOETUSES LEFT HORN
DF RIGHT = NUMBER OF DEAD FOETUSES RIGHT HORN

Table 7

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STUDY NO. 629 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN NZW-RABBITS

ID.NO = 4317

TABLE: 7 CONTINUED 2

FEMALES

		NF LTTR	POIL (%)	LF LEFT	LF RIGHT	LF LTTR	SEX-MLS	SEX-FEM
CONTROL	MEAN	0.23	8.039	3.5	4.7	8.23	3.9	4.3
	SEM	0.11	2.031	0.3	0.3	0.44	0.3	0.4
	N	22	22	22	22	22	20	20
4 MG/KG/DAY	MEAN	0.45	13.854	3.7	3.5	7.20	3.6	3.4
	SEM	0.17	3.424	0.4	0.3	0.54	0.4	0.5
	N	20	20	20	20	20	18	18
12 MG/KG/DAY	MEAN	0.19	9.955	3.2	3.9	7.10	3.9	3.0
	SEM	0.11	3.261	0.4	0.4	0.58	0.4	0.4
	N	21	21	21	21	21	20	20
36 MG/KG/DAY	MEAN	0.63	20.240	3.7	3.1*	6.84	3.8	3.4
	SEM	0.28	6.390	0.5	0.5	0.73	0.5	0.4
	N	19	19	19	19	19	18	18

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)
NF LTTR = NUMBER OF DEAD FOETUSES PER LITTER
LF LEFT = NUMBER OF LIVE FOETUSES LEFT HORN
LF LTTR = NUMBER OF LIVE FOETUSES PER LITTER
SEX-FEM = FOETAL SEX:NUMBER OF FEMALES
POIL = POST IMPLANTATION LOSS
LF RIGHT = NUMBER OF LIVE FOETUSES RIGHT HORN
SEX-MLS = FOETAL SEX:NUMBER OF MALES

Table 7
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EIVS/TMD

STUDY NO. 629 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN NZW-RABBITS ID.NO = 4319

TABLE: B AUTOPSY FINDINGS AND LITTER DATA, TOTAL VALUES PER GROUP

F E M A L E -

		IS GROUP	ER GROUP	LR GROUP	LF GROUP	LE GROUP	MAF
CONTROL	TOTAL	205.0	8.0	4.0	5.0	181.0	9.
	N	23	23	23	22	22	22
4 MG/KG/DAY	TOTAL	160.0	9.0	6.0	9.0	144.0	3.
	N	20	20	20	20	20	20
12 MG/KG/DAY	TOTAL	177.0	7.0	5.0	4.0	149.0	4.
	N	23	22	22	21	21	21
36 MG/KG/DAY	TOTAL	166.0	17.0	3.0	12.0	130.0	15.
	N	20	20	20	19	19	18

STATISTICS: ANOVA + DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

IS GROUP - NUMBER OF IMPLANTATION SITES PER GROUP
LR GROUP = NUMBER OF LATE RESORPTIONS PER GROUP
LF GROUP = NUMBER OF LIVE FOETUSES PER GROUP
ER LTTR = NUMBER OF EARLY RESORPTIONS PER GROUP
DF LTTR = NUMBER OF DEAD FOETUSES PER GROUP
MAF = NO.MACROSCOPICALLY VISIBLE ALTERED FOETUSES

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Table 9
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CIVB/IND

IB.NO = 5742

STUDY NO. 629 ORAL EMBRYOTOXICITY/TERATOGENICITY STUDY WITH AN AQUEOUS CYANAMIDE SOLUTION IN NZW-RABBITS

TABLE: 9 ORGAN WEIGHTS AND FOETUS WEIGHTS AND LENGTHS. MEAN VALUES PER LITTER BY DAM

FEMALES

		OVARY (GRAMS)	UTERUS-G (GRAMS)	UTERUS-E (GRAMS)	APLW (GRAMS)	AFW (GRAMS)	AFL (CM)
CONTROL	MEAN	0.944	481.64	69.87	4.84	40.48	10.0
	SEM	0.043	23.22	3.38	0.18	1.27	0.1
	N	22	22	22	22	22	22
4 MG/KG/DAY	MEAN	0.940	436.20	63.28	4.40	40.68	10.0
	SEM	0.054	27.62	3.64	0.20	1.00	0.1
	N	20	20	20	20	20	20
12 MG/KG/DAY	MEAN	1.013	429.43	61.09	4.49	42.26	10.1
	SEM	0.045	27.82	2.21	0.24	1.16	0.1
	N	21	21	21	21	21	21
36 MG/KG/DAY	MEAN	0.892	407.44	60.83	4.00	38.77	9.7
	SEM	0.053	34.01	3.48	0.25	1.92	0.2
	N	19	18	18	18	18	18

STATISTICS: ANOVA & DUNNETT TESTS * P<0.05 ** P<0.01 TWO SIDED (EXP.UNIT = ANIMAL)

OVARY = OVARY WEIGHT
UTERUS-E = EMPTY UTERUS WEIGHT
AFW = AVERAGE FOETAL WEIGHT
UTERUS-G = GRAVID UTERUS WEIGHT
APLW = AVERAGE PLACENTAL WEIGHT
AFL = AVERAGE FOETAL LENGTH

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Table 8
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