5-19-91

FILE COPY

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

19 APR 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Health Effects Division (HED) Peer Review Committee
Draft Document on CYANAZINE

FROM: Esther Rinde, Ph.D.
Manager, HED Carcinogenicity Peer Review
Science Analysis Coordination Branch
Health Effects Division (H7509C)

TO: Addressees

Attached for your review is the draft document of the Peer Review Committee on CYANAZINE, prepared by Dr. George Ghali. Please provide your comments on the draft document and return to me no later than May 3, 1991. If a reply is not received by that time, we will presume that you concur and have no comments.

Should you need a few extra days for a thorough review, please let us know that your comments are forthcoming.

ADDRESSEES

P. Fenner-Crisp
W. Burnam
R. Engler
R. Hill
K. Baetcke
B. Beliles
M. Copley
K. Dearfield
J. Du
B. Fisher
G. Ghali
H. Pettigrew
J. Parker
E. Rinde
W. Sette
M. Van Gemert
Y. Woo
G. Burin
W. Dykstra
R. Gardner

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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: DRAFT Peer Review of Cyanazine (Bladex)

CAS No.: 21725-46-2
EPA Chem. Code: 100101
CFR No.: 180.307

FROM: William Dykstra, Ph.D.
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

and

George Z. Ghali, Ph.D. G. Ghali 4/19/91
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Connie Childress, PM 74
Reregistration Branch
Special Review and Reregistration Division (H7508C)

Tom Moriarity
Section II
Special Review Branch
Special Review and Reregistration Division (H7508C)

and

Robert Taylor, PM 25
Fungicide-Herbicide Branch
Registration Division (H7505C)

The Health Effects Division Peer Review Committee convened on March 20, 1990 to discuss and evaluate the weight of the evidence on Cyanazine with particular emphasis on its carcinogenic potential. The Committee concluded that Cyanazine should be classified as a Group C, possible human carcinogen. Quantification of human risk, using a low-dose extrapolation model (Q^*1), was also recommended.

2

A. Individual in Attendance

1. Peer Review Committee (Signature indicates concurrence with the peer review unless otherwise stated.)

William L. Burnam _____

Reto Engler _____

Karl Baetcke _____

Marcia Van Gemert _____

Esther Rinde _____

Hugh Pettigrew _____

George Ghali _____

2. Peer Review Members in Absentia (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Penny Fenner-Crisp _____

Richard Hill _____

Kerry Dearfield _____

Jean Parker _____

William Sette _____

Robert Beliles _____

Marion Copley _____

Yin-Tak Woo _____

Julie Du _____

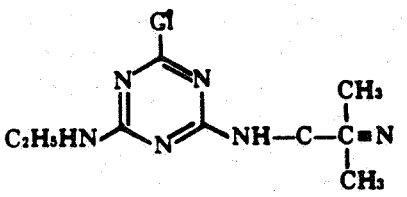
3. Scientific Reviewers (Committee or noncommittee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

William Dykstra _____

Roger Gardner _____

B. Background Information:

Cyanazine [2-((4-chloro-6-(ethylamino)-5-triazine-2-yl)amino)-2-methyl-propionitrile] is a symmetrical triazine used as a preemergence or postemergence selective herbicide. Cyanazine is registered for use on corn, cottonseed, sorghum, and wheat. Tolerances for Cyanazine residues range from 0.05 to 0.2 part per million (ppm). A registration standard on Cyanazine was completed in 1984.



Cyanazine (Bladex)

C. Material Reviewed:

Material available for review by the Committee consisted of a summary document addressing the issue and related toxicology information prepared by Dr. William Dykstra, data evaluation records for the chronic toxicity, carcinogenicity study in the rat and carcinogenicity study in the mouse, spontaneous neoplastic lesion in the Crl: CD rats, and a toxicology one-liners for cyanazine.

D. Evaluation of Carcinogenicity Data:

1. Bogdanffy, M.S. (1990). Combined chronic toxicity/oncogenicity study with Cyanazine in rats. Unpublished report prepared by Haskell Laboratory and submitted by E.I. du Pont de Nemours and Company. Study No. 23-90, Report dated May 11, 1990. MRID No. 415099-02.

   a. Experimental Design

      Groups of 52 male and 52 female young Sprague-Dawley rats were fed cyanazine technical at the concentrations of 0, 1, 5, 25, or 50 ppm in the diet for 2 years. Additionally, 10 animals per sex per group were used as a satellite group for interim sacrifice at 12 months.

   b. Considerations of Dose Selection

The highest dose tested was considered to be adequate for carcinogenicity testing based upon a decrease body weight gain of about 14 percent in both males and females in the first 3 months of the study. However, the Committee indicated that animals could have tolerated higher doses.

Dose selection for this study was primarily based on: a) decrease body weight gain observed at 25 ppm (HDT) in males (9.6%) and females (9.4%) during the first 3 months in Carworth Farm E strain rats in a chronic toxicity carcinogenicity study completed in 1973 (Accession No. 251954, -55, -56); b) decreased body weight gain observed at 50 ppm (HDT) in males (9.4%) and females (9.6%) during the first 24 weeks of a 2-year chronic toxicity/carcinogenicity study completed in 1970 in Carworth Farm E strain rats (Accession No. 251949 thru 251953). Because of major deficiencies these two studies were not considered in the weight of the evidence determination.

c. Microscopic Pathology

1) Nonneoplastic - Generally, there were no nonneoplastic lesions that could be attributed to treatment. However, there was three lesions of concern. These lesions were a) granulocytic hyperplasia of bone marrow in males (significant trend, $p = 0.0187$); b) extramedullary hematopoiesis of the spleen in males (significant trend, $p = 0.0230$ and significant pairwise comparison at 50 ppm, $p = 0.0359$); and c) demyelination of the sciatic nerve in females (significant trend, $p = 0.0125$). Historical control data for these lesions are required to determine whether these are treatment-related. These lesions have not been reported with other triazine herbicides.

2) Neoplastic - There was a statistically significant increase in the malignant mammary gland tumors (adenocarcinoma, carcinosarcoma, and fibrosarcoma) in females of the 5, 25, and 50 ppm groups, with a statistically significant positive trend ($p = 0.0034$).

Table 1: Incidences of Malignant Mammary Gland Tumors (Adenocarcinoma, Carcinosarcoma, and Fibrosarcoma) in Female Rats: Fisher's Exact Test/Cochran-Armitage Trend Test

Dose: (ppm) (mg/kg/day)	0.0 0.0000	1.0 0.0500	5.0 0.2500	25.0 1.2500	50.0 2.5000
	5/58 (9)	7/61 (11)	13/60 (22)	20/62 (32)	16/62 (26)
	p=0.0034**	p=0.4172	p=0.0420*	p=0.0012**	p=0.0116*

Malignant dataset: Excludes animals that die before week 48
First tumor occurred at 48 weeks in the control group.

There was no increase in the incidence of benign mammary gland tumors as shown in Table 2.

Table 2: Incidences of Benign Mammary Gland Tumors (Adenoma, Fibroadenoma, and Fibroma) in Female Rats: Fisher's Exact Test/Cochran-Armitage Trend Test

Dose: (ppm) (mg/kg/day)	0.0 0.0000	1.0 0.0500	5.0 0.2500	25.0 1.2500	50.0 2.5000
	23/58 (40)	26/61 (43)	24/60 (40)	20/62 (32)	27/62 (44)
	p=0.4508	p=0.4435	p=0.5596	p=0.2566	p=0.4026

Benign dataset: Excludes animals that die before week 48
First tumor occurred at 53 weeks in the 1 ppm dose group.

The incidences of malignant tumors of the mammary glands were outside the historical control range (10.1 to 22.0% with an average of 17.9%).

2. Gellatly, J. (1981). A two year feeding study of Bladex in the mouse, unpublished report prepared by Shell Toxicology Laboratory, submitted by Shell Chemical Company, Report No. 1493, dated December 1981. EPA Accession No. 247295 thru 298.

a. Experimental Design:

Five groups of 50 CD-1 mice/sex/dose were fed cyanazine technical in the diet for 2 years at the concentrations of 10, 25, 250, or 1000 ppm. The control group cosisted of 100 animals/sex. The average diet analysis for concentra-tions over the 2 years were 10.0 $\pm$ 4.5, 24.8 $\pm$ 4.3, 240 $\pm$ 5.2, and 983 $\pm$ 5.5 ppm.

b. Considerations of Dose Selection:

The 250 ppm dose was considered to be adequate for carcinogenicity testing based upon statistically significant decrease (10 to 23 percent) in body weight gain ranging up to 14 percent in males and 23 percent in females during the entire study. At 1000 ppm, palatability problems were seen as significant excess food spillage by both sexes during the entire study.

c. Microscopic Pathology:

Nonneoplastic - The nonneoplastic lesions observed included: centrilobular parenchymal hypertrophy of the liver, diffuse cortical tubular dilation of the kidney, acute and subacute myocarditis, basal myocardial fibrosis, and prominent hematopoiesis of bone marrow in male mice. In females, the nonneoplastic lesions included: hepatic parenchymal atrophy, diffuse cortical epithelium vacuolation of the kidney, basal and nonbasal myocardial fibrosis, adrenal cortical lipid depletion, corpora calcification of brain stem, skin patchy ulceration, and prominent hematopoiesis of bone marrow.

Neoplastic - There was an increased incidence of hemangiosarcoma of the spleen in males at 10 ppm (8%) which was statistically significant when compared to controls. Hemangiosarcoma of the spleen did not occur at the mid-high and high dosage levels. For hemangiosarcomas at all sites, the incidence at 10 ppm males was 12% (out of which 4% was in the liver) compared to only 3% in the

control males. Historical control data from Tunstall Laboratories were not provided. Recent historical control data from other laboratories indicated that the range for the spontaneous incidence of hemangiomas/hemangiosarcoma occur spontaneously in this strain of mice may vary between 3.3 to 13.3%. For this reason and because of the lack of a clear dose-response relationship, it was concluded that the statistically significant incidence at 10 ppm is not compound-related.

E. Other Relevant Toxicology Information:

1. Mutagenicity:

Cyanazine induced forward mutation in a dose-related manner in repeat assays with and without metabolic activation in the L5178Y/TK cells. Cyanazine was positive for in vitro unscheduled DNA synthesis in repeat assays in rat hepatocytes. On the other hand, Cyanazine was reported to be negative for gene mutation in CHO/HPRT cells and for chromosomal aberrations in human lymphocyte cultures.

2. Developmental Toxicity:

a. Sprague-Dawley Rats

Oral administration of 30 mg/kg/day resulted in maternal body weight reductions and increased incidences of piloerection (RTI #31T-2564). The maternal systemic NOEL was therefore determined at 3 mg/kg/day. No developmental toxicity effects were noted up to and including the highest dose used (30 mg/kg/day).

b. Fischer-344 Rats

In the first study (WRC RIR-180) dose levels of 0, 1, 2.5, 10, and 25 mg/kg/day were administered orally to pregnant rats during the period of major organogenesis (days 6 to 15). Diaphragmatic hernia was noted at all dosage levels tested and anophthalmia/microphthalmia was observed in fetuses of the 25 mg/kg dosage level. However, in the absence of a dose-response relationship and historical control data, the toxicological significance of these findings could not be ascertained. To fully evaluate the nature of these findings as well as the survivability of the affected fetuses, a teratology study with a postnatal

phase was requested by the Agency and later conducted by Argus Research Lab. (#619-002).

In the second Fisher-344 rat study (Argus Research No. 619-002), dose levels of 0, 5, 25, and 75 mg/kg were used. Dams were treated orally during the period of major organogenesis (days 6 to 15) and a postnatal investigation was included in this study. Dose-related increases in maternal clinical manifestations were noted at all dose levels and the maternal NOEL was established at < 5 mg/kg/day (LDT). Alterations in skeletal ossification sites were noted in all groups. However, other developmental effects were observed only at the 75 mg/kg (anophthalmia/microphthalmia, dilated brain ventricles, cleft palate, and abnormalities of the diaphragm) and the 25 mg/kg (abnormalities of the diaphragm) dosage levels. The study was classified as Core-Minimum Data with a developmental toxicity NOEL at 5 mg/kg/day.

c. New Zealand Rabbits - Oral Administration

Technical Bladex was given to pregnant rabbits at 0, 1, 2, and 4 mg/kg/day (Tunstall Lab. No. 221/81) from days 6 to 18 of gestation. Maternal systemic toxic signs were evidenced by anorexia, weight loss, death, and abortion noted at the 2 and 4 mg/kg dosage levels. Alterations in skeletal ossification sites, decreased litter size, and increases in postimplantation loss were also observed at the 2 and 4 mg/kg dosage levels. Developmental effects (domed cranium, dilated brain ventricles, anophtalmia/micro-phthalmia, and thoracoschisis) were associated with the 4 mg/kg dosage level. Based upon these findings, both the developmental toxicity and maternal NOELs were established at 1 mg/kg/day and the study was classified as Core-Minimum Data.

d. New Zealand Rabbits - Dermal Administration

A dermal developmental toxicity study was conducted with the Bladex 4L formulation in pregnant rabbits (WIL No. 93002). All animals were exposed to 100, 300, 600, or 1000 mg/kg during days 6 to 18 of gestation. Each day, neck collars were affixed for 6 hours during the exposure period. Significant decreases in maternal weights and food consumption associated with increased incidences of deaths

and abortions were noted in all treated groups. Due to a high incidence of maternal loss, the number of litters available for examination was substantially reduced and both the maternal and developmental toxicity NOELs could not be ascertained with confidence. The study was classified as Core-Supplementary Data.

In a repeat study (WIL No. 93003), dosage levels of 96, 283, 573, and 955 mg/kg were applied dermally to pregnant rabbits during the period of major organogenesis (days 6 to 18 of gestation). All animals were restrained in stocks during the daily exposure period (6 hours) and wore a neck collar for the rest of the day. Dermal irritation was noted in all treated animals but significant body weight depressions and food reductions were found only in dams exposed to 283, 573, or 955 mg/kg/day. Evidence of a developmental effect was not observed in the treated groups except for increased incidences of skeletal variations at the 955 mg/kg dosage level. Under the conditions of this study, the maternal NOEL was established at < 96 mg/kg (LDT). A developmental toxicity NOEL was demonstrated at 573 mg/kg and the study was classified as Core-Minimum Data.

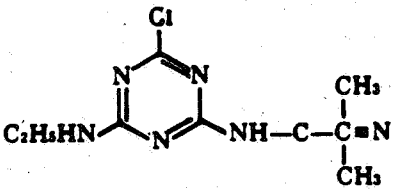
It should be noted that the registrant has fulfilled all regulatory requirements for teratogenicity testing with Cyanazine (Bladex) in two species.

3. Reproductive Toxicity:

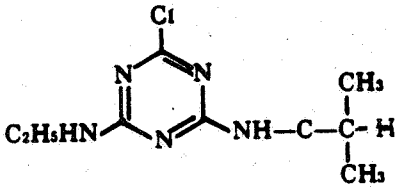
In a two-generation rat reproduction study, the NOEL for reproductive toxicity is 3.8 mg/kg/day with a LEL for reproductive toxicity of 11.2 mg/kg/day based on decreased pup viability and decreased mean pup body weight during lactation of dams on a diet with 75 ppm. The LEL for systemic toxicity is less than or equal to 1.8 mg/kg/day (LDT) based on decreased body weight of males (not statistically significant) and females ($p < 0.01$) of F1 adults at various time periods throughout the study. The study was acceptable as Core-Minimum Data.

4. Structure Activity Relationship

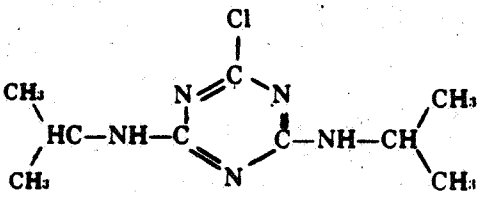
Cyanazine is structurally related to simazine, atrazine, propazine, and terbutryn, the structures of which are shown below.



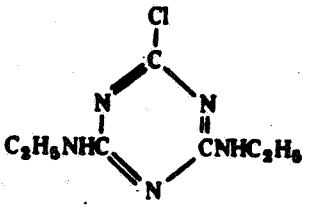
Cyanazine



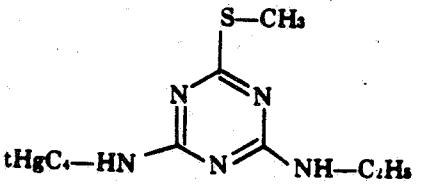
Atrazine



Propazine



Simazine



Terbutryn

Figure 1: Cyanazine and structurally related compounds

Simazine was associated with statistically significant increases in carcinomas of the pituitary gland (at the HDT) and mammary gland (at the mid (100 ppm) and highest dose) in the female Sprague-Dawley rat, when fed in the diet at doses up to 1000 ppm. The incidence of mammary gland tumors at the HDT was well outside the range reported for historical controls at the testing facility. The incidence of pituitary gland tumors was just outside the historical control range; however, it exceeded (considerably) the incidences reported for 6 out of 7 studies. The pituitary tumors in the female rats were fatal with a possibly accelerated onset, and the mammary carcinomas also contributed to the increased mortality at the HDT, according to the study authors. Simazine was not associated with increases in neoplasms when fed in the diet to CD-1 mice, at doses up to 4000 ppm. The study was considered to have been adequately conducted. Simazine was classified as a group C carcinogen. Quantification of human risk using a low-dose extrapolation model ($Q1^*$) was recommended.

Administration of atrazine to female Sprague-Dawley rats was associated with a statistically significant increase in mammary gland fibroadenomas at 1000 ppm, in mammary gland adenocarcinomas (including two carcinosarcomas at the HDT) at 70, 500, and 1000 ppm, and in total mammary gland tumor-bearing animals at 1000 ppm. Each of these increases was associated with a statistically significant dose-related trend and was outside the high end of the historical control range. In addition, there was evidence for decreased latency for mammary gland adeno-carcinomas at the 12-month interim sacrifice. Atrazine was not carcinogenic when tested in the CD-1 mice.

Atrazine was negative in three acceptable assays for mutagenicity. Atrazine was not teratogenic in rats or rabbits and caused no reproductive toxicity in rats up to 1000 ppm. Atrazine was classified as a Group C carcinogen. Quantification of human risk using a low-dose extrapolation model ($Q1^*$) was recommended.

Propazine was negative for oncogenicity in the CD-1 mouse but caused a statistically significant increase in mammary gland tumors in female CD rats.

Propazine has been found to be positive for mutagenicity in V79 Chinese hamster cells both with and without metabolic activation. However, the response was weaker in the presence of metabolic activation. It was negative in a nucleus anomaly assay and in a DNA repair assay in rat hepatocytes. Propazine has been classified as a Group C,

When administered in the diet to female Charles River CD rats, terbutryn induced a statistically significant increase in combined mammary gland adenomas and adenocarcinomas and in combined hepatocellular adenomas and carcinomas. In males, terbutryn induced an increase in combined thyroid follicular cell adenomas and carcinomas and in testicular interstitial cell adenomas. Terbutryn is negative for oncogenicity in the CD-1 mouse. Terbutryn is not mutagenic in the Ames Salmonella assay and the micronucleus assay and does not cause chromosomal aberrations in vivo in hamsters. Terbutryn has been classified as a group C carcinogen.

5. Metabolism

Orally administered radio-labeled cyanazine was rapidly metabolized in the rat. The major portion of the dose was excreted in four days in the urine (40.7%) and feces (47.2%) suggesting that a portion of the material may be excreted via the bile and may undergo entrohepatic circulation. The excretion rate of radioactivity in the feces was slower than that of the urine. Only 3% of the administered dose remained in the animal after four days. The total recovery of the radioactivity was 93.2%. The absence of radioctive carbon dioxide from the ring-labeled cyanazine in the expired gases suggests that the triazine ring remains intact. On the other hand, the large amount of radioactive carbon dioxide expired during the metabolism of the ethyl-labled cyanazine suggests that N-deethylation is a major metabolic pathway. Major urine metabolites include N-acetyl-S-[4-amino-6-(1-methyl-1-cyanoethylamino)-s-triazinyl]-L-cysteine and 2-chloro-4-amino-6-(1-methyl-1-cyanoethylamino)-s-triazine (Huson, D. et al. (1970). J. Ag. Food Chem., Vol. 18, No. 3, pg 507-512).

F. Weight of the Evidence:

The Committee considered the following facts to be of importance in the weight-of-the-evidence determination of the carcinogenic potential of Cyanazine.

1. Dietary administration of Cyanazine to Sprague-Dawley rats for 2 years was associated with a statistically significant increase in the incidence of malignant mammary gland tumors (adenocarcinoma, carcinosarcoma, fibrosarcoma) in females at three dose levels. There was also a statistically significant ($p = 0.0034$) positive trend.

   The incidences of malignant mammary gland tumors in the study were more than the historical control range of 10.1 to 22.0 percent (average 17.9%). Malignancy was more prevalent in the treated groups when compared to controls.

   The treatment did not alter the spontaneous tumor profile in males. The high dose tested was considered adequate for carcinogenicity testing based upon body weight-gain reduction of about 14% in both males and females in the first 3 months of the study. However, the Committee concluded that animals could have tolerated higher doses.

2. Dietary administration of Cyanazine to CD-1 mice for 2 years did not alter the spontaneous tumor profile in this strain of mice. The Committee considered that the mid-high dose tested (250 ppm) to be adequate for carcinogenicity testing based on reduction in body weight gain of 10 to 23 percent in males and females during the entire study. At the high dose tested (1000 ppm), palatability problems were evident.

3. Cyanazine induced forward mutation in a dose-related manner with and without metabolic activation in the L5178Y/TK cells. Cyanazine was also positive for <u>in vitro</u> unscheduled DNA synthesis in rat hepatocytes. On the other hand, Cyanazine was reported to be negative for gene mutation in CHO/HPRT cells and for chromosomal aberration in human lymphocyte cultures.

4. Cyanazine is considered a developmental toxin and causes several types of malformations in rats and rabbits.

5. Cyanazine is structurally related to other triazines such as simazine, atrazine, propazine, and terbutryn known to induce cancer in experimental animals.

G. Classification:

Considering criteria contained in EPA Guidelines (FR 51:33992-34003, 1986] for classifying a carcinogen, the Committee concluded that the data available for Cyanazine provided evidence to classify the chemical as a Group C, possible human carcinogen. This classification was based upon:

1. Statistically significant increase in the incidences of malignant mammary gland tumors (adenocarcinoma, carcinosarcoma, fibrosarcoma) in female Sprague-Dawley rats. This increase in the incidence of malignant tumors showed a statistically significant ($p = 0.0034$) positive trend. The incidences of malignant mammary gland tumors were outside the range of historical control. Malignancy was more prevalent in the treated groups when compared to controls.

2. Induction of forward mutation in the L5178Y/TK cells with and without metabolic activation, and positive response in the in vitro UDS assay with rat hepatocytes.

3. Structural similarity to other triazine herbicides known for their carcinogenic potential.

Quantification of human cancer risk, using a low-dose extrapolation model ($Q1^*$), was also recommended. This decision was based upon the fact that Cyanazine induced malignant tumors and malignancy was more prevalent in treated animals when compared with controls. Additionally, Cyanazine is genotoxic and structurally similar to other carcinogens. The calculation of the potency factor will be based on malignant mammary gland tumors.