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

30 JUL 1991

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MEMORANDUM

SUBJECT: Peer Review of Cyanazine (Bladex)

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

FROM: William Dykstra, Ph.D. William Dykstra 5/21/91
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

and

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Science Analysis and Coordination Branch
Health Effects Division (H7509C)

TO: Lois Rossi, Chief
Reregistration Branch
Special Review and Reregistration Division (H7508C)

and

Janet Auerbach, Chief
Special Review Branch
Special Review and Reregistration Division (H7508C)

The Health Effects Division Peer Review Committee convened on March 20, 1991 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.

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.)

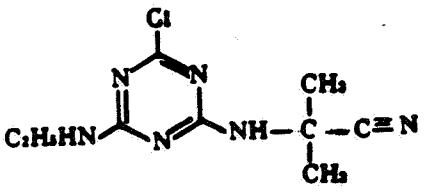
Penny Fenner-Crisp
Richard Hill
John Quest
Kerry Dearfield
Jean Parker
William Sette
Robert Beliles
Marion Copley
Yin-Tak Woo
Julie Du

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 lesions 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 decreased 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 probably have tolerated higher doses.

Dose selection for this study was primarily based on: a) decreased 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, at this time, be attributed to treatment. However, there were 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 malignant mammary gland tumors (adenocarcinoma and carcinosarcoma) in females of the 25, and 50 ppm groups, with a statistically significant positive trend ($p = 0.0049$).

Table 1: Incidences of Malignant Mammary Gland Tumors (Adenocarcinoma and Carcinosarcoma) 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)	12/60 (22)	20/62 (32)	15/62 (24)
	p=0.0049**	p=0.4172	p=0.0661	p=0.0012**	p=0.0193*

Malignant data set: Excludes animals that died 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 data set: Excludes animals that died before week 48
First tumor occurred at 53 weeks in the 1 ppm dose group.

The incidences of malignant tumors of the mammary glands at 25 and 50 ppm were outside the historical control range (10.1 to 22.7% 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:

      Four groups of 50 CD 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 consisted of 100 animals/sex. The average diet analysis for concentrations over the 2 years were 10.0 ± 4.5, 24.8 ± 4.3, 240 ± 5.2, and 983 ± 5.5 ppm.

   b. Considerations of Dose Selection:

      The 250 ppm dose was considered to be adequate for carcinogenicity testing based upon a 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 (1%). 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 in CD-1 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 was 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 mouse lymphoma L5178Y/TK cell gene mutation assay. Cyanazine was positive for in vitro unscheduled DNA synthesis in repeat assays in rat hepatocytes. Negative results were reported for gene mutation in CHO/HPRT assay and for chromosomal aberrations in human lymphocyte cultures. This testing satisfies the minimal testing for the three categories of mutagenicity testing. Based on the positive results, additional testing is required to examine the effects or interaction with germ cells.

2. Developmental Toxicity:

Cyanazine was not associated with developmental effects when tested orally up to 30 mg/kg/day in SD rats. In Fischer 344 rats, diaphragmatic hernia was noted at dosage levels as low as 1 mg/kg/day. However,in the absence of a dose-response relationship and appropriate historical control data, the toxicological significance of these findings could not be ascertained. In a second study in Fischer 344 rats, alterations in skeletal malformations were noted in all groups (5, 25, 75 mg/kg/day). Other developmental effects observed at 75 mg/kg/day included anophthalmia/microphthalmia, dilated brain ventricles, cleft palate, and diaphragm abnormalities. Abnormalities of the diaphragm were observed also at 25 mg/kg/day.

In New Zealand rabbits, oral administration of Cyanazine to pregnant animals from days 6 to 18 of gestation was associated with alterations in skeletal ossification sites, decreased litter size, and increases in postimplantation loss at the middle (2 mg/kg/day) and high (4 mg/kg/day)

dose levels. Developmental effects associated with the 4 mg/kg/dose level included domed cranium, dilated brain ventricles, anophthalmia/microphthalmia, and thoracoschisis. Dermal application of Cyanazine to the skin of New Zealand rabbits resulted in no developmental effects except for increased incidences of skeletal variations at the highest dose tested (955 mg/kg/day).

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. Metabolism

Generally, s-triazine metabolism in animals involves mainly reaction of the C-2 substitutes, conjugation, N-dealkylation, and side chain modification. Deamination and ring cleavage are not considered of any significance in animal metabolism of s-triazines. When parent s-triazines are fed to animals, degradation essentially ends at the stage of 2-hydroxy-4-amino-6-alkylamino derivatives or at 4,6-diamino compounds with an intact C-2 substituent. Both types can be directly cleared by the kidneys. Therefore, stages of further degradation occur in animals in significant amounts only when these compounds are fed directly in the form of plant metabolites.

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 intrahepatic 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 radioactive 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-labeled 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 (Hutson, D. et al. (1970). J. Ag. Food Chem., Vol. 18, No. 3, pg 507-512).

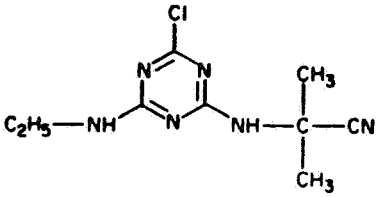
Unlike other 2-chloro-4,6-bis-alkylaminotriazines, the major urinary metabolite of cyanazine is the mercapturic acid conjugate indicating that glutathione conjugation is a major metabolic pathway for cyanazine. Apparently, the presence of the cyano group in the N-substituent favors the glutathione conjugation over the N-dealkylation indirectly indicating that cyanazine can generate more electrophilic arylating agent than other 2-chloro-4,6-bis-alkylaminotriazines. This is consistent with finding that cyanazine yielded a more positive genotoxic response.than other 2-chloro-4,6-bis-alkylaminotriazines.

5. Structure Activity Relationship

Cyanazine is structurally related to simazine, atrazine, propazine, and terbutryn, the structures of which are shown in Figure 1.

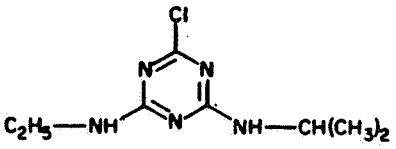
Except for terbutryn, all these triazines are substituted diamino-s-triazines which have a chlorine. Terbutryn has a thiomethyl group on carbon 2 instead of the chlorine.

The remarkable stability of s-triazine derivatives can be explained by the electronic configuration of the heterocyclic ring which resembles that of benzene. However, essential differences exist in the electronic configuration between the s-triazine and benzene as a consequence of the greater electronegativity of the nitrogen atoms as compared to that of the carbon atoms. Therefore the electrons in the s-triazines ring are in the vicinity of the nitrogen atoms rather than being evenly distributed over the whole ring. A polar mesomeric form that bears an additional pair of unshared electrons on the nitrogen atoms will therefore contribute, to a certain degree, to the actual structure of the s-triazine molecule.



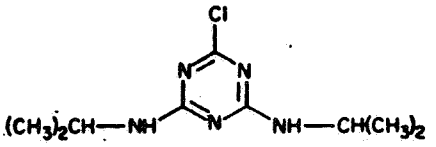
2-Chloro-4-ethylamino-6-(1-cyano-1-methylethylamino)-s-triazine

Cyanazine



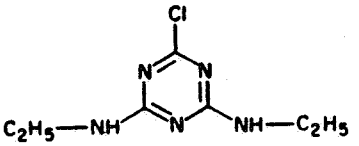
2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

Atrazine



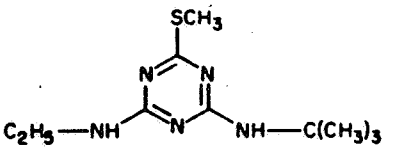
2-Chloro-4,6-bis(isopropylamino)-s-triazine

Propazine



2-Chloro-4,6-bis(ethylamino)-s-triazine

Simazine



2-Methylthio-4-ethylamino-6-tert-butylamino-s-triazine

Terbutryn

Figure 1: Cyanazine and structurally-related compounds

As a result the aromatic character of the s-triazine is less pronounced than that of benzene.

The same delocalization effect in combination with inductive and mesomeric effects exerted by the substituents at the three carbon atoms greatly influences the reactivity of the s-triazines. The relative electron deficiency of the ring carbon atoms makes them susceptible to nucleophilic attack. This attack is facilitated when electron withdrawing substituents such as chlorine are attached to the carbon atoms, and is impeded when the electron density of the aromatic system is increased by electron-supplying substituents such as amino groups.

Unlike other 2-chloro-4,6-bis-alkylaminotriazines, Cyanazine has a cyano group in the N-substituent. Apparently, the presence of the cyano group in the N-substituent favors the glutathione conjugation over the N-dealkylation, indirectly indicating that cyanazine can generate more electrophilic arylating agent than other 2-chloro-4,6-bis-alkylaminotriazines. This is consistent with finding that cyanazine yielded a more positive genotoxic response than other 2-chloro-4,6-bis-alkylaminotriazines and thus the positive carcinogenic response at dose levels lower than those required to invoke such response with other 2-chloro-4,6-bis-alkylaminotriazines.

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 is negative in a submitted Salmonella assay, but there are positive and negative data in published literature. Positive results are reported in the mouse lymphoma, Drosophila sex-

linked recessive lethal and cell transformation assays. 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 adenocarcinomas 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 although there are some positive results reported in published literature including mouse bone marrow aberrations and a mouse dominant lethal test. 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 carcinogenicity 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, possible human carcinogen.

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 Salmonella assay and the micronucleus assay and does not cause chromosomal aberrations in vivo in hamsters. Terbutryn has been classified as a group C, possible human carcinogen.

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 and carcinosarcoma) in females at two dose levels. There was also a statistically significant ($p = 0.0049$) positive trend.

   The incidences of malignant mammary gland tumors at 25 and 50 ppm were more than the historical control range of 10.1 to 22.7 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 probably could have tolerated higher doses.

2. Dietary administration of Cyanazine to CD 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 in vitro unscheduled DNA synthesis in rat hepatocytes. This genotoxic activity provides support for a carcinogenicity concern for heritable effects. Testing for interaction with germ cells needs to be performed. In two other acceptable tests, Cyanazine was reported to be negative for gene mutation in CHO/HPRT cells and for chromosomal aberrations 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 mammary gland cancer in experimental animals. However, unlike other 2-chloro-4,6-bis-alkylaminotriazines, the major urinary metabolite of cyanazine is the mercapturic acid conjugate indicating that glutathione conjugation is a major metabolic pathway for cyanazine. Apparently, the presence of the cyano group in the N-substituent favors the glutathione conjugation over the N-dealkylation indirectly indicating that cyanazine can generate more electrophilic arylating agent than other 2-chloro-4,6-bis-alkylaminotriazines. This is consistent with finding that cyanazine yielded more positive genotoxic response than other 2-chloro-4,6-bis-alkylaminotriazines, and might explain also the induction of mammary gland cancer at even lower doses than those used with other triazines.

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) 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. Evidence of positive genotoxic activity in the mouse lymphoma gene mutation assay and for unscheduled DNA synthesis in 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.

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

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