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08/20/91

PEER REVIEW FILES

CHEMICAL NAME: Cyanazine (Bladex)
CASWELL NO.: 188C
CAS NO.: 21725-46-2
REVIEWER: Dykstra

CURRENT AGENCY DECISION

C; 8.4 x 10-1 (HED)

TUMOR TYPE / SPECIES

Mammary gland tumors (adenocarcinoma, carcinosarcoma); Sprague-Dawley rat (F)

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. / /	2. / /	2.
1. 03/07/91	1. 03/20/91	1. 07/30/91	1. C; 8.4 x 10-1

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. / /	1.

QUALITATIVE RISK ASSESSMENT DOCUMENT	QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
3. / /	3. / /	1. / /
2. / /	2. 07/08/91	
1. / /	1. 05/13/91	

MISCELLANEOUS:

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

30 JUL 1991

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

George Z. Ghali, Ph.D. G. Ghali 5.21.91
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.

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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.)

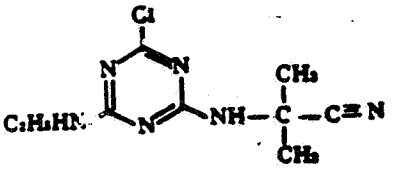
Penny Fenner-Crisp
Richard Hill —
John Quest
Kerry Dearfield
Jean Parker
William Sette
Robert Beliles
Marion Copley
Yin-Tak Woo
Julie Du

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).

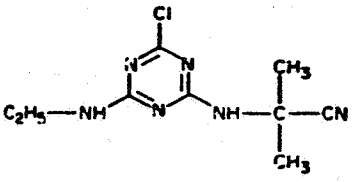
Unlike other 2-chloro-4,6-bis-alkylaminotriazines, the major urinary metabolite of cyanazine is the mercapturic acid conjugate indicating that glutathione conjugation is a major metabolic pathway for cyanazine. Apparently, the presence of the cyano group in the N-substituent favors the glutathione conjugation 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.

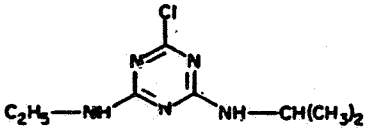
Except for terbutryn, all these triazines are substituted diamino-s-triazines which have a chlorine. Terbutryn has a thiomethyl group on carbon 2 instead of the chlorine.

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.



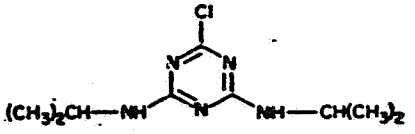
2-Chloro-4-ethylamino-6-(1-cyano-1-methylethylamino)-s-triazine

Cyanazine



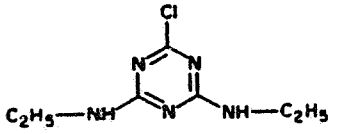
2-Chloro-4-ethylamino-6-isopropylamino-s-triazine

Atrazine



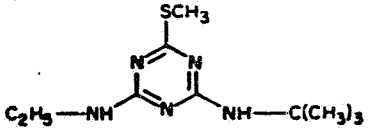
2-Chloro-4,6-bis(isopropylamino)-s-triazine

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

13 MAY 1991

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Cyanazine (188C), Atrazine (63) and Simazine (738) Quantitative Risk Assessment Comparisons on Malignant Mammary Gland Tumors only in Rats.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Bernice Fisher 5/13/91

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Esther Rinde, Ph.D., Acting Section Head E.R.
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)
and
Reto Engler, Ph.D., Chief
Scientific Analysis & Corrdination Branch
Health Effects Division (H7509C)

Estimated+ $Q_1^*(mg/kg/day)^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	$Q_1^*(mg/kg/day)^{-1}$ Rat	$Q_1^*(mg/kg/day)^{-1}$ In Human Equiv.++
Cyanazine	Carcinoma, Adenocarcinoma & Fibrosarcoma	1.66×10^{-1}(a)	8.8×10^{-1}
Atrazine[1]	Carcinoma	1.72×10^{-2}(b)	9.2×10^{-2}
Simazine	Carcinoma	2.25×10^{-2}(b)	1.2×10^{-1}

+ Based on results from Statox computer program
++Derived by the use of surface area correction - $(\text{Human Wt.}/\text{Rat Wt.})^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-tumor Multi-Stage Model (Weibull83)

1 HED's previous estimate of Q_1^* was 2.2×10^{-1} based upon both benign & malignant mammary gland tumors. For the puposes of comparison with Cyanazine & Simazine, only malignant tumors were used in the estimation of the unit risk, Q_1^*.

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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Cyanazine (1 3C), Atrazine (63) and Simazine (740) Quantitative Risk Assessment Comparisons on Malignant Mammary Glan: Tumors only in Rats. Revised Comparisons as of July, 1991.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Bernice Fisher 7/8/91

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Kerry L. Dearfield, Ph..D., Acting Section Head
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Kerry L Dearfield 7.8.91

and

Reto Engler, Ph.D., Chief
Scientific Analysis & Coordination Branch
Health Effects Division (H7509C)

HED's previous estimate of cyanazine's Q_1^* of 8.8×10^{-1} was based upon malignant mammary gland tumors including fibrosarcomas. For comparative purposes with atrazine and simazine, malignant tumors including adenocarcinomas, carcinomas and carcinosarcomas only are used in the estimation of the unit risk, Q_1^*.

Animals with fibrosarcomas in the cyanazine study are excluded from the group for the estimate of Q_1^*. The reason for this exclusion is due to advice given by Dr. Brennecke (HED's consultant in pathology) that fibrosarcomas do not originate from epithelial cell tissues as do the carcinomas. The carcinosarcomas, which originate from both the epithelial and mesenchymal cell tissues, found in both the atrazine and cyanazine mammary gland malignant tumor data can be retained for the estimate of Q_1^*.

cc Kathy Pearce SRRD

Table on Estimated[+] Q_1^*(mg/kg/day)$^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	Q_1^*(mg/kg/day)$^{-1}$ Rat	In Human Equiv.[++]
Cyanazine	Carcinosarcomas & Adenocarcinoma	1.59×10^{-1}(a)	8.4×10^{-1}(c)
Atrazine	Adenocarcinoma & Carcinosarcoma	1.72×10^{-2}(b)	9.2×10^{-2}(c)
Simazine	Carcinoma	2.25×10^{-2}(b)	1.2×10^{-1}(c)

[+] Based on results from Statox computer program
[++] Derived by the use of surface area correction - (Human Wt./Rat Wt.)$^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-Tumor Multi-Stage Model (Weibull83)
(c) Cyanazine - This Q_1^* is the estimate to be used for Risk Characterization.
Atrazine - This Q_1^* is the estimate for comparative purposes only of the three chemical compounds and is not the one that is used for Risk Characterzation (actual estimate used is 2.2×10^{-1} based upon both benign and malignant mammary gland tumors).
Simazine - This Q_1^* is the estimate that has been and is still being used for Risk Characterization.

008521

FILE COPY

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

13 MAY 1991

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Cyanazine (188C), Atrazine (63) and Simazine (7[illegible]) Quantitative Risk Assessment Comparisons on Malignant Mammary Gland Tumors only in Rats.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Esther Rinde, Ph.D., Acting Section Head
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)
and
Reto Engler, Ph.D., Chief
Scientific Analysis & Corrdination Branch
Health Effects Division (H7509C)

Estimated+ Q_1^* $(mg/kg/day)^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	Q_1^* $(mg/kg/day)^{-1}$ Rat	Q_1^* $(mg/kg/day)^{-1}$ In Human Equiv.++
Cyanazine	Carcinoma, Adenocarcinoma & Fibrosarcoma	1.66×10^{-1}(a)	8.8×10^{-1}
Atrazine[1]	Carcinoma	1.72×10^{-2}(b)	9.2×10^{-2}
Simazine	Carcinoma	2.25×10^{-2}(b)	1.2×10^{-1}

+ Based on results from Statox computer program
++Derived by the use of surface area correction - $(\text{Human Wt.}/\text{Rat Wt.})^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-tumor Multi-Stage Model (Weibull83)

1 HED's previous estimate of Q_1^* was 2.2×10^{-1} based upon both benign & malignant mammary gland tumors. For the puposes of comparison with Cyanazine & Simazine, only malignant tumors were used in the estimation of the unit risk, Q_1^*.

008521

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

FILE COPY

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Cyanazine (188C), Atrazine (63) and Simazine (740)
Quantitative Risk Assessment Comparisons on Malignant Mammary Gland Tumors only in Rats. Revised Comparisons as of July, 1991.

From: Bernice Fisher, Biostatistician
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)

Bernice Fisher 7/8/91

To: Karl Baetcke, Ph.D., Chief
Toxicology Branch I (IRS)
Health Effects Division (H7509C)

Thru: Kerry L. Dearfield, Ph.D., Acting Section Head
Science Support & Special Review Section
Science Analysis & Coordination Branch
Health Effects Division (H7509C)
and
Reto Engler, Ph.D., Chief
Scientific Analysis & Coordination Branch
Health Effects Division (H7509C)

Kerry Dearfield 7.8.91

HED's previous estimate of cyanazine's Q_1^* of 8.8×10^{-1} was based upon malignant mammary gland tumors including fibrosarcomas. For comparative purposes with atrazine and simazine, malignant tumors including adenocarcinomas, carcinomas and carcinosarcomas only are used in the estimation of the unit risk, Q_1^*.

Animals with fibrosarcomas in the cyanazine study are excluded from the group for the estimate of Q_1^*. The reason for this exclusion is due to advice given by Dr. Brennecke (HED's consultant in pathology) that fibrosarcomas do not originate from epithelial cell tissues as do the carcinomas. The carcinosarcomas, which originate from both the epithelial and mesenchymal cell tissues, found in both the atrazine and cyanazine mammary gland malignant tumor data can be retained for the estimate of Q_1^*.

cc Kathy Pearce SRRD

Table on Estimated[+] $Q_1^*(mg/kg/day)^{-1}$ for Cyanazine, Atrazine and Simazine in Sprague-Dawley Female Rats

	Tumors in the Mammary Gland	$Q_1^*(mg/kg/day)^{-1}$ Rat	In Human Equiv.[++]
Cyanazine	Carcinosarcomas & Adenocarcinoma	$1.59x10^{-1}$(a)	$8.4x10^{-1}$(c)
Atrazine	Adenocarcinoma & Carcincsarcoma	$1.72x10^{-2}$(b)	$9.2x10^{-2}$(c)
Simazine	Carcinoma	$2.25x10^{-2}$(b)	$1.2x10^{-1}$(c)

[+] Based on results from Statox computer program
[++]Derived by the use of surface area correction - $(\text{Human Wt.}/\text{Rat Wt.})^{1/3}$

(a) Multi-Stage Model (Global86)
(b) Time-to-Tumor Multi-Stage Model (Weibull83)
(c) Cyanazine - This Q_1^* is the estimate to be used for Risk Characterization.
Atrazine - This Q_1^* is the estimate for comparative purposes only of the three chemical compounds and is not the one that is used for Risk Characterzation (actual estimate used is $2.2x10^{-1}$ based upon both benign and malignant mammary gland tumors).
Simazine - This Q_1^* is the estimate that has been and is still being used for Risk Characterization.

[redacted] 008521

Duplicate

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

MAR 7 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review on Cyanazine

FROM: Esther Rinde, Ph.D. E.R.
Manager, Carcinogenicity Peer Review
Health Effects Division (H7509c)

TO: Addressees

Attached for your review is a package on Cyanazine prepared by Dr. William Dykstra.

A meeting to consider the classification of Cyanazine is scheduled for Wednesday March 20, 1991, at 10:00 am in Room 821, CM2.

Addressees

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

25

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008521

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Peer Review of Cyanazine

40 CFR 180.307
TOX Chem. No.: 188C

FROM: William Dykstra, Ph.D., D.A.B.T. William Dykstra 2/28/91
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

TO: Esther Rinde, Ph.D.
Manager, Peer Review for Oncogenicity
Science Analysis and Coordination Branch
Health Effects Division (H7509C)

THRU: Roger L. Gardner, Section Head 3/4/91
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

A. Background

Cyanazine is an S-triazine selective herbicide, preplant incorporated or used as preemergence and postemergence and is registered for use on corn, cottonseed, sorghum, and wheat. Tolerances range from 0.05 to 0.2 part per million (ppm).

The data base has been updated since the early 1980's. A Registration Standard was compiled in 1984.

It is requested that the Committee conduct a peer review of this compound and recommend a carcinogenicity classification for cyanazine.

B. Documentation Received

The key study is a 1990 Rat Chronic Toxicity/ Oncogenicity Study at Haskell Laboratories.

The remainder of the cyanazine data base consists of a 2-year mouse oncogenicity study, mutagenicity studies, developmental toxicity studies in rats and rabbits, a 2-generation rat reproduction study, and a 1-year dog study. The rat metabolism study is currently a data gap.

C. Chemical Information

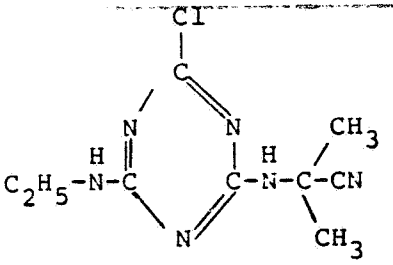
Registrant: E.I. du Pont de Nemours & Company

Chemical Name: 2-((4-chloro-6-(ethylamino)-s-triazin-2-yl)amino)-2-methylpropionitrile

Synonyms: SD 15418, WL 19805

TOX Chem. No.: 188C

Structure:



D. Evaluation of Oncogenicity Studies

Two oncogenicity studies are presented for consideration--the first in rats and the second in mice.

Reference: Matthew S. Bogdanffy. Combined Chronic Toxicity/Oncogenicity Study with Cyanazine (INR-1957). Two-Year Feeding Study in Rats. Study No. 23-90, May 11, 1990, MRID No. 415099-02.

Randomized groups of 52 male and 52 female young (age 38 days) Sprague-Dawley rats were fed cyanazine technical in the diet at dosages of 0, 1, 5, 25, and 50 ppm for 2 years. Additionally, 10/sex/group were sacrificed at 12 months. The total per sex/group was 62 rats.

High-dose male rats had significantly improved survival in comparison to controls. Survival of other treated groups of male rats and all treated groups of female rats were comparable in survival to their respective controls.

At the 50 ppm dose, male and female rats had significant decreases in body weight gain (up to 14% in males and females) in comparison to controls. These significant body weight gain decreases are considered as evidence that an MTD was employed in the study. Food consumption was only slightly decreased at 50 ppm in both sexes. There were no significant differences between controls and treated male and female groups in ophthalmologic findings, hematology, clinical chemistries, urinalysis, and organ weights.

A statistically significant increase in the incidence of female rats with mammary gland masses was observed in the 25 and 50 ppm groups between 1 year and terminal sacrifice. These masses were correlated histologically with the significant increase in adenocarcinomas in those groups.

At dosages of 5, 25, and 50 ppm, cyanazine technical was associated with significantly increased incidences of total malignant tumors of the mammary gland in female rats. The tumors occurred in 5/58, 7/61, 13/60, 20/62, and 16/62 tumor-bearing rats at dosages of 0, 1, 5, 25, and 50 ppm, respectively. The following tables show the occurence of malignant mammary gland tumors

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: ADENOCARCINOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	1/3	0/2	0/0	0/0	0/1	1/6
53- 78	0/13	1/19	2/19	4/19	4/19	11/89
79-106	4/44	6/41	10/41	15/43	11/42	46/211
TOTALS	5/60	7/62	12/61	19/62	15/62	58/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: CARCINOSARCOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	0/19	0/19	1/19	0/19	1/89
79-106	0/44	0/41	0/41	0/43	0/42	0/211
TOTAL	0/60	0/62	0/61	1/62	0/62	1/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: FIBROSARCOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	0/19	0/19	0/19	0/19	0/89
79-106	0/44	0/41	1/41	0/43	2/42	3/211
TOTAL	0/60	0/62	1/61	0/62	2/62	3/307

First tumor occurred at 48 weeks in dose group # 1

DOSE mg/ (mg/kg/day	0.0000	0.0500	0.2500	1.2500	2.5000
	5/58	7/61	13/60	20/62	16/62
	(9)	(11)	(22)	(32)	(26)
	p=0.0034 **	p=0.4172	p=0.0420*	p=0.0012**	p=0.0116*

Reference: J.B.M. Gellatty. A Two-Year Feeding Study of Bladex in Mice. Study No. 1493. December 1981. Accession No. 247295-298.

Randomized groups of 100 male and 100 female control CD-mice and 50 male and 50 female CD-mice at dosages of 10,

25, 250, and 1000 ppm were fed cyanazine technical in their diets for 2 years.

There were no compound-related effects on survival in treated male and female mice in comparison to their controls.

No compound-related tumors were observed at dosages up to 1000 ppm [highest dose tested (HDT)].

At 250 ppm, there were significant (10 to 23%) decreases in body weight gain ranging up to 14 percent in males and 23 percent in females during the entire study. Part of the decreased body weight gain was due to decreased food consumption, although the remainder reflects the direct toxicity of cyanazine.

At 1000 ppm, palatability problems were seen as significant excess food spillage by both sexes during the entire study.

The no-observable-effect level (NOEL) for clinical signs, gross necropsy findings, increased incidences of histological effects, and clinical pathology results was 25 ppm.

The NOEL for decreased relative kidney weight to body weight was 10 ppm.

The NOEL for systemic toxicity may be 10 ppm [lowest dose tested (LDT)], although 3 to 7 percent body weight gain decreases were observed in females during most of the study.

The incidence of hemangiosarcoma of the spleen in males was 1/100 (1%), 4/50 (8%)*, 2/50 (4%), 0/50, and 0/50 for the 0, 10, 25, 250, and 1000 ppm groups, respectively. The incidence of total number of tumorbearing male mice with hemangiosarcomas was 3, 12*, 4, 2, and 2 percent for the 0, 10, 25, 250, and 1000 ppm groups, respectively (*$p < 0.05$).

The lack of dose-response, the occurrence of a historical range for CD-1 male mice in the open literature up to 13.3 percent, and the lack of increase in this tumor type in treated females (control females had 2/100) suggested that this tumor type was not compound-related at the 10 ppm level.

E. Additional Toxicological Information

1. Mutagenicity

Study	Reported Results	TB Evaluation
Gene Mutation in L5178Y/TK Cells	Positive for induced forward mutation in a dose-responsive fashion in repeat assays with/ without activation (viable dose range = 0.5 to 500 ug/mL).	ACCEPTABLE
DNA Damage/Repair (UDS) in HPC	Positive for unscheduled DNA synthesis in repeat assays in rat hepatocytes treated in vitro (viable dose range = 1 to 100 uM).	ACCEPTABLE
Ames Assay	Although reported as negative for inducing reversions in Salmonella TA strains exposed up to 5000 ug/plate (causing 50% toxicity), many procedural and reporting deficiencies exist.	UNACCEPTABLE
Gene Mutation in CHO/HPRT Cells	Negative for inducing mutation in repeat assays in nonactivated and activated Chinese hamster ovary cells treated up to cytotoxic limits of solubility (1.4 mM).	ACCEPTABLE
Chromosomal Aberrations in Human Lymphocyte Cultures	Negative in repeat assays with human lymphocytes exposed in the presence/absence of activation to cytotoxic dose levels (250 to 350 ug/mL).	ACCEPTABLE

2. Metabolism

A data gap exists for this study in rats. The following information was contained in the Atrazine Peer Review Document:

> In rats, 89% of labelled cyanazine is eliminated within 4 days, 42% in urine and 47% in feces. The major metabolic pathways are dechlorination and deethylation.

F. Developmental and Reproductive Effects

1. Developmental Toxicity Studies

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, anophthalmia/microphthalmia, 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.

2. Reproduction Study

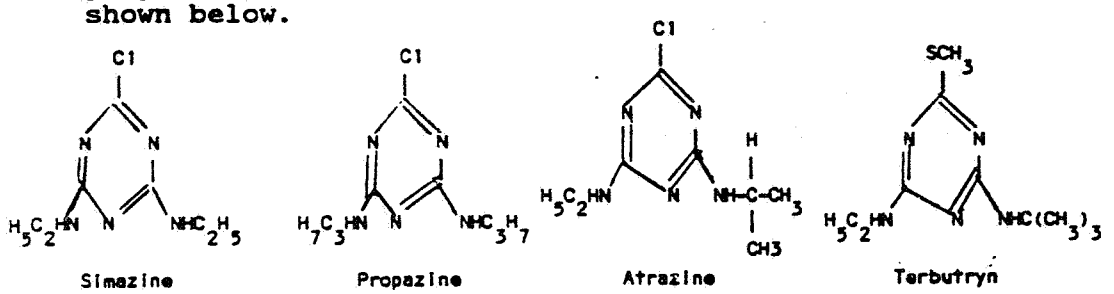
In a two-generation rat reproduction study, the NOEL for reproductive toxicity is 3.8 mg/kg/day with a LEL for reproductive toxicity of 11.2 mg/kg/day based on decreased pup viability and decreased mean pup body weight during lactation of dams on a diet with 75 ppm. The LEL for systemic toxicity is less 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.

3. Chronic Dog Study

The NOEL for systemic toxicity was 25 ppm (both sexes). The LEL for systemic toxicity is 100 ppm based on reduced body weights and body weight gains, elevated platelet counts, reduced levels of total protein, albumin and calcium in males and females. There were also slight, not statistically significant, decreases in spleen weights and increases in liver weights in the females and increases in liver weights and decreases in testes weights in the males. No gross or microscopic findings related to treatment were noted. 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.



a. Simazine

Simazine is rapidly metabolized in the rat. Eighty-six percent of the labeled compound is excreted within 14 hours in the urine and feces. 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 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 classified as a group C carcinogen with a Q_1*

b. Atrazine

Complete metabolism studies are not available; however, atrazine has been shown to be excreted mainly in the urine.

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.

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.

In the CD-1 mouse, atrazine was negative in a 91-week study. Atrazine was classified as a Group C carcinogen without quantification.

c. Propazine

Forty-two percent of ^{14}C-propazine was eliminated in the urine and 28 percent in the feces. Mostly unchanged propazine was found in the feces. Hydroxypropazine was identified in both urine and feces.

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 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 classified as a group C carcinogen.

d. Terbutryn

Eighty-five percent of ring-labeled ^{14}Cterbutryn is excreted within 72 hours in the urine (39%) and feces (46%) of rats. The major metabolic pathways are desulfuration, N-deethylation, and S-demethylation.

Terbutryn is not mutagenic in the Ames *Salmonella* assay and the micronucleus assay and does not cause chromosomal aberrations *in vivo* in hamsters.

Terbutryn is negative for oncogenicity in the CD-1 mouse.

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 has been classified as a group C carcinogen.

64776:I:Dykstra:LHED-7:KEVRIC:02/14/91:03/13/91:DD:WO:CL
R:64777:Dykstra:LHED-7:KEVRIC:02/20/91:03/19/91:aw:EK:CL
R:64779:Dykstra:LHED-7:KEVRIC:02/27/91:03/26/91:DD:WO:EK:DD
M. Bishop corrected 3/4/91

Fishers' Exact Test/Cochran-Armitage trend test
RA# 152; CYANAZINE; FEMALE; RAT, STUDY--

Benign dataset: Excludes animals that die before week 48

MAMMARY GLAND; ADENOMA - Evaluated
MAMMARY GLAND; FIBROADEN - Evaluated
MAMMARY GLAND; FIBROMA - Evaluated

First tumor occured at 53 weeks in dose group # 2

DOSE(mg/kg/day)	0.0500	0.2500	1.2500	2.5000
	26/61	24/60	20/62	27/62
	(43)	(40)	(32)	(44)
p= ?	p= 0.4435	p= 0.5596	p= 0.2566	p= 0.4026

	CHI-SQUARE	DF	P VALUE
LINEAR TREND (Ho: no trend)	0.0049	1	0.4508 (one-sided)
DEPARTURE (Ho: linear)	2.0336	3	0.5692 (two-sided)

Fishers' Exact Test/Cochran-Armitage trend test
RA# 152; CYANAZINE; FEMALE, STUDY--

Malignant dataset: Excludes animals that die before week 48

MAMMARY GLAND; ADENOCARCINOMA - Evaluated
MAMMARY GLAND; CARCINOMA - Evaluated
MAMMARY GLAND; FIBROSARCOMA - Evaluated

First tumor occured at 45 weeks in dose group # 1

DOSE(mg/kg/day)	0.0000	0.0500	0.2500	1.2500	2.5000
	7/61	13/60	20/62	16/62	
	(11)	(22)	(32)	(26)	
p= ?**	p= 0.4172	p= 0.0420*	p= 0.0012**	p= 0.0116*	

	CHI-SQUARE	DF	P VALUE
LINEAR TREND (Ho: no tr	7.2959	1	0.0034** (one-sided)
DEPARTURE (Ho: linear)	7.3263	3	0.0611 (two-sided)

Fishers' Exact Test/Cochran-Armitage trend test
RA# 152; CYANAZINE; F[illegible], STUDY--

Combined dataset: Excl[illegible]imals that die before week 48

MAMMARY GLAND; ADENOC[illegible] - Evaluated
MAMMARY GLAND; ADE[illegible]
MAMMARY GLAND; FI[illegible]uated
MAMMARY GLAND; FIBR[illegible]
MAMMARY GLAND; CARC[illegible] - Evaluated
MAMMARY GLAND; FI[illegible]ated

First tumor occured at [illegible] in dose group # 1

DOSE(mg/kg/day)	0.0500	0.2500	1.2500	2.5000
	30/61	32/60	30/62	36/62
	(49)	(53)	(48)	(58)
	p= 0.3853	p= 0.2298	p= 0.4179	p= 0.1025

	[illegible]-SQUARE	DF	P VALUE	
LINEAR TREND (Ho: no [illegible]	.3276	1	0.1239	(one-sided)
DEPARTURE (Ho: line[illegible]	[illegible]717	3	0.7631	(two-sided)

TUMOR REPORT FOR RA# 152; CYANAZINE; FEMALE; RAT

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: ADENOCARCINOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	1/3	0/2	0/0	0/0	0/1	1/6
53- 78	0/13	1/19	2/19	4/19	4/19	11/89
79-106	4/44	6/41	10/41	15/43	11/42	46/211
TOTAL	5/60	7/62	12/61	19/62	15/62	58/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: ADENOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	2/19	1/19	0/19	0/19	3/89
79-106	2/44	4/41	2/41	4/43	2/42	14/211
TOTAL	2/60	6/62	3/61	4/62	2/62	17/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: FIBROADENOMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	1/13	3/19	3/19	2/19	4/19	13/89
79-106	21/44	17/41	18/41	17/43	21/42	94/211
TOTAL	22/60	20/62	21/61	19/62	25/62	107/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: FIBROMA

	DOSE GROUP					
INTERVALS	1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	0/19	0/19	0/19	0/19	0/89
79-106	1/44	0/41	1/41	0/43	0/42	2/211

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TOTAL	1/60	0/62	1/61	0/62	0/62	2/307

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TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: CARCINOSARCOMA

INTERVALS	DOSE GROUP 1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	0/19	0/19	1/19	0/19	1/89
79-106	0/44	0/41	0/41	0/43	0/42	0/211
TOTAL	0/60	0/62	0/61	1/62	0/62	1/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: FIBROSARCOMA

INTERVALS	DOSE GROUP 1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	0/3	0/2	0/0	0/0	0/1	0/6
53- 78	0/13	0/19	0/19	0/19	0/19	0/89
79-106	0/44	0/41	1/41	0/43	2/42	3/211
TOTAL	0/60	0/62	1/61	0/62	2/62	3/307

TISSUE TYPE: MAMMARY GLAND
TUMOR TYPE: HYPERPLASIA

INTERVALS	DOSE GROUP 1	2	3	4	5	TOTAL
1- 26	0/0	0/0	0/1	0/0	0/0	0/1
27- 52	2/3	2/2	0/0	0/0	1/1	5/6
53- 78	9/13	16/19	8/19	13/19	15/19	61/89
79-106	23/44	18/41	11/41	18/43	24/42	94/211
TOTAL	34/60	36/62	19/61	31/62	40/62	160/307

(6)

Reviewed by: William Dykstra William Dykstra 10/18/90
Section I, Toxicology Branch I - IRS (H7509C) Roger Gardner 2/11/91 008521
Secondary Reviewer: Roger Gardner, Acting Section Head
Section I, Toxicology Branch I - IRS (H7509C)

DATA EVALUATION REPORT

Study Type: 83-5 - Combined Chronic Toxicity/Oncogenicity - Rat

TOX Chem. No.: 188C

Accession Number: N/A

MRID No.: 415099-02 (2 Volumes)

Test Material: INR-1957 (96% purity)

Synonyms: Cyanazine technical

Study Number: Haskell Laboratory Project No. 23-90

Sponsor: E.I. du Pont de Nemours and Company

Testing Facility: Haskell Laboratory

Title of Report: Combined Chronic Toxicity/Oncogenicity Study with Cyanazine (INR-1957) 2-Year Feeding Study in Rats.

Author: Matthew S. Bogdanffy

Report Issued: May 11, 1990

Conclusions

At dosages of 5, 25, and 50 ppm, cyanazine technical was associated with increased incidences of 20, 30, and 23 percent, respectively, which were outside available Charles River historical controls (0-16%) for adenocarcinoma mammary gland tumors in female Sprague-Dawley rats. The total number of tumor-bearing animals with adenocarcinomas was 5, 7, 12, 19, and 14 for 0, 1, 5, 25, and 50 ppm, respectively. The total number of tumor-bearing animals with malignant tumors (all types) was 5, 7, 13, 20, and 16 for the 0, 1, 5, 25, and 50 ppm group, respectively.

Statistically, there was a significant trend for adenocarcinoma gland tumors and the pairwise comparison using "crude proportions" for the 5, 25, and 50 ppm dose levels were 0.0535, 0.0021, and 0.013, respectively. It appears from the data that cyanazine is a adenocarcinoma (malignant) mammary gland carcinogen in Sprague-Dawley rats.

Classification:

Core Supplementary. Additional data are needed.

Special Review Criteria (40 CFR 154.7):

A carcinogenic Special Review criterion has been exceeded by this study.

A. Materials:

1. Test Compound - INR-1957; Description not stated; Batch #H-16,489; Purity 96.0%; Contaminants: List in CBI appendix.

2. Test Animals - Species: Rat; Strain: Sprague-Dawley; Age: 38 days; Weight: Males - 32.5 to 63.3 g; Females - 36.7 to 62.5 g.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups and housed individually.

Test Group	Dose in Diet (ppm)	Main Study 24 Months Male	Main Study 24 Months Female	Interim Sac. 12 Months Male	Interim Sac. 12 Months Female
1 Control	0	52	52	10	10
2 Low (LDT)	1	52	52	10	10
3 Mid (MDT)	5	52	52	10	10
4 Mid (MDT)	25	52	52	10	10
5 High (HDT)	50	52	52	10	10

2. Diet Preparation - Diet was prepared weekly and stored at refrigerated temperature. Samples of treated food were analyzed for stability and concentration at test days -1, 34, 181, 363, and 728.

Results - Stability studies showed results which ranged from 82 to 108 percent of nominal concentrations. Homogeneity analysis performed at the beginning, test day 34, and end of study showed adequate distribution of cyanazine in the diet. Analyses of samples from concentration at the various dosage levels and at various times showed diets were prepared within 17 percent of nominal concentrations at all times.

3. Animals received food (Rodent Chow #5002) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Analysis of variance, followed by Dunnett's test when significant for body weight, body weight gain, organ weights, and clinical laboratory data. Clinical observations were analyzed by Fisher's Exact test with the Bonferroni correction and the Cochran-Armitage test for trend. Tumor incidence was analyzed by the Fisher Exact test and the Cochran-Armitage test for trend. Survival probabilities were

estimated with the Kaplan-Meier procedure. Significance was judged at $p < 0.05$.

5. Quality assurance was performed routinely and both a signed statement for GLP adherence by the lab and Quality Assurance documentation signed by Kathleen C. Reed (May 3, 1990) were submitted.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality. Additionally, at each weighing, careful clinical examinations were performed for each rat.

With respect to toxic signs in males, there was a dose-related (trend significant) increase in hyperactivity which was statistically significant at the high dose. The number of hyperactive rats was 12, 17, 17, 24, and 34 for the 0, 1, 5, 25, and 50 ppm groups, respectively. Hyperactivity was not observed in females. However, the hyperactivity in males may be compound-related. The NOEL for hyperactivity is 5 ppm.

A significantly decreased trend in ruffled fur occurred with males but not females. The incidences for this phenomenon in males were 0, 12, 7, 1, and 0 for the 0, 1, 5, 25, and 50 ppm groups, respectively. This is not considered compound-related. There were no treatment-related effects in males with respect to tissue masses or the medians for days-on-test when given masses were first observed. The number of tissue masses were 25, 27, 25, 23, and 28 in the 0, 1, 5, 25, and 50 ppm groups, respectively.

In female rats, there was a significant increase in palpable masses in the inguinal area at 50 ppm in comparison to controls. The incidence of palpable masses (together with the medians for days-on-test when given signs were first observed) was 38 (406), 38 (427), 42 (427), 40 (370), and 51 (343)* for the 0, 1, 5, 25, and 50 ppm groups.

Therefore, the NOEL for clinical signs in female rats is 25 ppm.

With respect to survival, high-dose male rats (50 ppm) survived significantly better than other treated groups and control rats, which was ascribed to the up to 16 percent decrease in body weight gain for these animals during the study.

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*$p < 0.05$.

The mortality summary for male rats is shown below:

Dose (ppm)	0	1	5	25	50
Total Rats					
At start	62	62	62	62	62
Interim kill	10	10	10	10	10
Terminal kill	17	20	18	20	29
Died on study	35	32	34	32	23*
Percent survival (0-721 days)	33	38	35	38	56

*$p < 0.05$.

Cyanazine did not have an effect on survival in female rats. The mortality summary for female rats is shown below:

Dose (ppm)	0	1	5	25	50
Total Rats					
At start	62	62	62	62	62
Interim kill	10	10	10	10	10
Terminal kill	21	26	25	23	29
Died on study	31	26	27	29	23
Percent survival (0-721 days)	40	50	48	44	56

2. Body Weight - The rats were weighed once per week for 6 months, then once every other week for the remainder of the study.

Results - Mean body weight in males was decreased up to 18 percent in the 50 ppm group between days 7 and 707. Body weight gain was also decreased during the 0 to 371-day period. An MTD was established at 50 ppm by the 14 percent decrease in body weight gain over the 0 to 91-day period. Body weight and body weight gain were decreased at 25 ppm during most of the first year of study (see the attached charts). These decreases were statistically significant at 50 and 25 ppm in females and 50 ppm in males.

Mean body weight and body weight gain in females decreased in the 50 ppm group up to 16 and 14 percent, respectively.

An MTD was established at 50 ppm based on the 14 percent decrease in body weight gain over the 0 to 91-day interval. At 25 ppm, mean body weight gain was decreased 11 percent during days 0 to 91. (See attached charts.)

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

   Results - Only slight decreases in daily food consumption ranging from 6 to 9 percent in 50 ppm males, 2 to 4 percent in 25 ppm males, 4 to 6 percent in 50 ppm females, and 3 to 5 percent in 25 ppm females were noted over the intervals evaluated. Food efficiency was decreased 10 to 22 percent in the 25 and 50 ppm males and 9 to 16 percent in the 25 and 50 ppm female groups. The decreases in food efficiency were largely due to the decreased body weight gain.

   Mean daily intake of compound over the 0 to 721-day interval was 0, 0.040, 0.198, 0.985, and 2.06 mg/kg, respectively, for the 0, 1, 5, 25, and 50 ppm male groups.

   In females over the 0 to 721-day interval, mean compound intake daily was 0.0, 0.053, 0.259, 1.37, and 2.81 mg/kg for the 0, 1, 5, 25, and 50 ppm groups, respectively.

4. Ophthalmological examinations were performed at pretest, 1-year interim sacrifice, and at the end of the study on all control and high-dose animals.

   Results - There were no compound-related ocular effects at the high dose in comparison to controls for male and female rats at the three ophthalmological examinations at a) pre-dosing; b) test day 351; and c) test day 722, according to J.M. Clinton, D.V.M.

5. Blood was collected at 3, 6, 12, 18, and 24 months for hematology and clinical analysis from 10/sex/dose animals. The CHECKED (X) parameters were examined.

   a. Hematology

X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB conc. (MCHC)
X	Platelet count*	X	Mean corpuscular volume (MCV)

   Results - There were no compound-related effects in either sex in hematology results at 3, 6, 12, 18, or 24 months.

The statistically significant increases in hemoglobin hematocrit, MCH, and MCHC at most doses at 12 months in male rats were due to the unusually low hematology values of a single control rat (animal #421980, which had values of 5.29 x 10^6/uL, 8.9 g/dl, and 31 percent for RBC hemoglobin, and hematocrit, respectively. Evaluation of the rat #421980 histopathology showed no unusually related findings. Other statistically significant differences at other times for male and female treated rats in comparison to controls were randomly distributed, not dose-related, and were not considered compound-related.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins (calculated)
X	Sodium	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
X	Creatinine kinase*		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		

Results - There were no compound-related effects in either sex in clinical chemistry results at 3, 6, 12, 18, or 24 months. The only consistent statistically significant findings in males were decreased creatinine kinase at 24 months in the 5, 25, and 50 ppm groups, respectively. These decreases are not considered toxicologically significant since they could not be correlated with histopathology or any organ toxicity ("Lower than normal values probably have no meaning, but reflect either small muscle mass, sedentary life style, or both." Clinical Guide to Laboratory Tests, N.W. Tietz, (1983) Saunders Press).

The occurrence of statistically significant increases in glucose values at 18 months in females at 5, 25, and 50 in comparison to controls and in sodium values in females at 3 months were not considered toxicologically significant since they were not time-related. Other singly occurring statistically significant clinical chemistry findings in male and female treated rats in comparison to controls were not considered compound-related, since they occurred randomly in time, were

not dose-related, and in females had returned to control ranges by 24 months. The 24-month findings in males have been previously discussed.

6. Urinalysis - Urine was collected from fasted animals at 3, 6, 12, 18, and 24 months. The CHECKED (X) parameters were examined.

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

Results - There were no compound-related effects in urinalysis in either sex at 3, 6, 12, 18, or 24 months. The values for control and treated rats were generally comparable and no time-related or dose-related trends or statistically significant pairwise comparisons that were considered toxicologically significant were observed.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

X		X			
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve* (sciatic)
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	X	Adrenals*
X	Cecum*	XX	Kidneys*	X	Lacrimal gland and Harderian gland
X	Colon*	X	Urinary bladder*	X	Mammary gland*
X	Rectum*	XX	Testes*	X	Parathyroids*
XX	Liver*	X	Epididymides	X	Thyroids*
	Gall bladder*	X	Prostate		Other
X	Pancreas*	X	Seminal vesicle	X	Bone*
	Respiratory	X	Ovaries	X	Skeletal muscle*
X	Trachea*	X	Uterus*	X	Skin
X	Lung*	X	Vagina	X	All gross lesions and masses
X	Nose				

Results:

a. Organ Weight

12 Months - There were no statistically significant changes in absolute or relative organ weights in male or female rats sacrificed at 12 months.

24 Months:

Males - The absolute weight of kidneys in the 50 ppm group was statistically significantly decreased and mean relative weights of the testes in the 50 ppm were significantly increased. The decrease in kidney weight can be correlated with the decrease in chronic glomerulonephropathy in high-dose male rates in comparison to the control and other dose groups and is not considered toxicologically significant. Similarly, there was a decrease in testicular atrophy in high-dose males, together with a decrease in male body weight, which undoubtedly led to the increased relative weight of the testes. This finding is not toxicologically significant.

Females - There were no statistically significant changes in absolute or relative organ weights in females at 24 months.

b. Gross Pathology

0 to 1 Year - No compound-related gross lesions were observed at statistically significant increases in males or females up to 1 year.

1 to 2 Years - A statistically significant increase in the incidence of female rats with mammary gland masses was observed in the 25 and 50 ppm groups between 1 year and terminal sacrifice. These masses were correlated histologically with the significant increase in adenocarcinomas in those groups. (The incidences of mammary gland tissue masses in female rates necropsied after 1 year were 24/49*, 29/50, 24/51, 37/52, and 39/51** in the 0, 1, 5, 25, and 50 ppm groups, respectively.)

*Number examined.
**$p < 0.05$.

c. Microscopic Pathology

1) Non-neoplastic - Generally, there were few non-neoplastic lesions that could be associated with treatment. The following Table I, with statistical analyses, shows the histological lesions which occurred at increased incidences or had significant trends. 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).

These lesions have not been reported with other triazine herbicides.

To judge the toxicological significance of these lesions with either a significant trend or a pairwise comparison, historical control data would be needed.

In the case of extramedullary hematopoiesis of the spleen, analysis of the results of hematology did not reveal any compensatory response to anemia in males. However, the increase in granulocytic hyperplasia of the bone marrow in male rats may be associated with the spleen phenomenon.

More alarming, perhaps, is the significant trend for demyelination of the sciatic nerve in females. Comparison of the grades of all of the three lesions between control and treated animals, especially high-dose animals, did not reveal any apparent shift in pattern to a more severe grade for the treated animals in comparison to controls.

Based on this observation (lack of increase in severity of grade), together with the historical control data requested, these lesions may not be of toxicological significance.

2) Neoplastic - The only compound-related neoplastic lesion occurred in the mammary gland of female rats as shown in Table II.

As can be seen from Table II, there is a statistically significant trend (p = 0.0043) for adenocarcinomas in treated rats and statistically

Table I - Non-Neoplastic Lesions in Male and Female Rats in 2-Year Cyanazine Rat Study

Males

Dose (ppm)	0	1	5	25	50
No. Examined	61	35	35	35	62
Bone Marrow, granulocytic hyperplasia	7	3	5	6	14
Percentage	11%	8.5%	14%	17%	23%
p =	0.0187*	0.4703	0.4594	0.3136	0.0806

Males

Dose (ppm)	0	1	5	25	50
No. Examined	62	40	41	41	62
Spleen, extramedullary hematopoiesis	24	16	21	21	35
Percentage	39%	40%	51%	51%	56%
p =	0.0230*	0.5295	0.1469	0.1469	0.0359*

Females (Day 370 to Day 736)

Dose (ppm)	0	1	5	25	50
No. Examined	49	23	28	28	51
Sciatic nerve, demyelination	4	0	2	1	9
Percentages	8%	0%	7%	3.5%	18%
p =	0.0125*	0.2059	0.6200	0.3968	0.1328

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Table II - 2-Year Cyanazine Rat Study Mammary Gland Tumors

Dose (ppm)	0	1	5	25	50
No. Examined[a]	60	62	61	62	62
Hyperplasia[b]					
Mild	6	8	5	5	8
Moderate	1	4	4	3	6
Severe	3	1	2	0	2
Total Hyperplasia	10 (16)	13 (21)	11 (18)	8 (13)	16 (31)
Fibroma	1 (2)	0	1 (2)	0	0
Fibroadenoma	14	15	10	6	15
Fibroadenoma, mult.	4	5	6	3	4
Total Fibroadenoma	18 (30)	20 (32)	16 (26)	9 (15)	19 (31)
Adenoma	1	3	2	1	1
Adenoma, mult.	1	0	0	0	0
Total Adenoma	2 (3)	3 (5)	2 (3)	1 (2)	1 (2)
Adenocarcinoma	3	5	7	15	8
Adenocarcinoma, mult.	2	2	5	4	6
Total Adenocarcinoma	5 (8)°	7 (11)	12 (20)	19 (31)**	14 (23)*
Trend:	p = 0.0043; p = .3813; p = 0.0535; p = 0.0021; p = 0.013				
Carcinosarcoma				1	
Fibrosarcoma			1		2
Total Number of Tumor-Bearing Rats	26 (43)	30 (48)	32 (52)	30 (48)	36 (58)

° = Significant trend (p = 0.0043)
* = $p < 0.05$.
** = $p < 0.01$.
[a] Based on histopathology sheets in volume 2 of the report
[b] Hyperplasia of tumor-bearing animals only.
Note: Each tumor-bearing animal was only counted once. The statistical analysis of total adenocarcinomas is based only on the "crude proportions" of number of animals examined and does not take into account possible survival disparity between groups. A complete statistical package will be prepared for the Peer Review.
(Numbers in parentheses are percentages)

significant pairwise comparisons for adenocarcinomas at 5 ppm ($p = 0.0535$), 25 ppm ($p = 0.0021$), and 50 ppm ($p = 0.013$). Additionally, there is one carcinosarcoma at 25 ppm, and one and two fibrosarcomas at 5 and 50 ppm, respectively, which were not introduced into the statistical computation.

Also, the statistical analysis is based on "crude proportions" and the p values will undoubtedly be of greater significance when a complete statistical package is prepared for Peer Review by the Biostatistics Team of SACB.

In reply to the study, the registrant offers the following "Historical controls":

INCIDENCE OF SPONTANEOUS PRIMARY MALIGNANT MAMMARY NEOPLASMS FROM 1 YEAR TO FINAL SACRIFICE IN CONTROL FEMALE Crl:CD®BR RATS FROM 2-YEAR FEEDING STUDIES AT HASKELL LABORATORY (1984-1989)

PATHOLOGY REPORT	ANIMALS PER GROUP	ANIMALS WITH MALIGNANT TUMORS (%)
20-84	69	7 (10.1)
43-85	66	15 (22.7)
2-86	66	10 (15.2)
9-86	59	12 (20.3)
10-87	60	9 (15.0)
10-88	60	13 (21.7)
63-89	59	13 (22.0)
102-89	47	8 (17.0)

Data includes control groups only. The number of animals with tumors represent the number of animals with a single or multiple tumor occurrence. Malignant tumors consist of adenocarcinomas and carcinosarcomas.

Total Animals in Historical Data Base = 486
Total Animals with Malignant Tumors = 87
Percent Animals with Malignant Tumors = 17.9%
Range of Spontaneously Occurring Malignant Tumors = 10.1 to 22.7%.

Additionally, the registrant calculates the malignant tumor incidence for the present study as shown below.

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INCIDENCE OF PRIMARY MALIGNANT MAMMARY NEOPLASMS IN FEMALE Crl:CD®BR RATS FROM 1 YEAR TO FINAL SACRIFICE IN THE CURRENT STUDY

GROUP	ANIMALS PER GROUP	ANIMALS WITH MALIGNANT TUMORS (%)
II	49	4 (8.2)
IV	50	6 (12.0)
VI	51	12 (23.5)*
VIII	52	18 (34.6)*
X	51	15 (29.4)*

An asterisk (*) indicates a significant difference from control group (Fisher's Exact Test) and a positive dose relationship (Cochran-Armitage Trend Test).

TB calculates the malignant tumor incidence for the study as follows:

Dose	Number In Group	Animals With Malignant Tumors
0	62	5 (8%)
1	62	7 (11%)
5	61	13 (21%)
25	61	20 (32%)
50	62	16 (29%)

It can be clearly seen that the registrant's tumor count is less than TB's count. When the TB Biostatistics Team perform survival disparity, the correct number of rats per group will be less than presently shown. However, the animals with malignant tumors calculated by TB will not change.

The registrant states that the concurrent control percentage of malignant tumors (8%) is below the registrant's historical control range. By comparing the concurrent treated groups to this "artificially" low concurrent control group, the p values have become exaggerated ($p < 0.05$).

TB's reply to this line of thinking is that perhaps all concurrent groups, control and treated, are too low.

In any event, the percentage incidences for malignant tumors in the cyanazine study, even using the "crude proportion" denominators, exceed the registrant's historical controls at 25 and 50 ppm (32%, 29%, vs. 22.7% "registrant's highest control value"). Additionally, the incidence of malignant tumors at 5 ppm is 21 percent (using an "crude proportion" denominator where n = 62) which is closely approximate to the registrant's highest control value.

A more correct comparison of the "crude proportions" adenocarcinoma incidences in the cyanazine study would be to compare them to a large data base which does not artifically cull the number of animals examined (as Haskell has done).

The Charles River Laboratories Data Base provides such a source of information.

The following information has been taken from the Charles River Breeding Laboratories Publication "Spontaneous Neoplastic Lesions in the Crl:CD®BR Rat":

"Common Study Parameters

"Data from eleven groups of control animals are presented in Tables 1-9. All studies had the following parameters in common:

- o They ran for 24 months
- o The diet was Purina 5001 (Rodent Lab Chow) or 5002 (Certified Rodent Chow)
- o Rats were housed individually in hanging wire mesh cages
- o Lesions tabulated were assumed to be primary site tumors only
- o The in-life completion dates range from 1977 to 1985
- o CD® rats were supplied from Charles River production facilities at Wilmington, MA, Portage, MI, or Kingston, NY."

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	No. Examined	No. Tumors	Mean	Range
Mammary Gland	843			
adenoma (NOS)		35	4.1	0-13.3
cystadenoma		4	0.5	0- 4.2
papillary adenoma		1	0.1	0- 1.2
intraductal papilloma		1	0.1	0- 1.5
adenocarcinoma (NOS)		63	7.4	0-16.0
ductular adenocarcinoma		1	0.1	0- 1.3
carcinoma (NOS)		21	2.5	0-19.1
fibroma		2	0.2	0- 1.3
fibroadenoma		287	33.9	14.6-58.1
fibrosarcoma		1	0.1	0- 1.4
hemangiopericytoma		1	0.1	0- 1.1
mammary neoplasia (NOS)		1	0.1	0- 1.1

EXPANDED TABLE OF MAMMARY TUMORS
IN FEMALE CD® RATS: 24 MONTHS

	GROUP										
TUMOR	A	B	C	D	E	F	G	H	I	J	K
N =	79	78	85	74	75	96	90	54	68	74	75
Adenoma (NOS)	1	5	1	4	2	5	12	1	3	1	--
Adenocarcinoma	--	4	11	4	3	15	3	--	--	12	11
Carcinoma (NOS)	--	--	--	--	1	--	--	7	13	--	--
Fibroadenoma	30	25	24	21	18	14	34	27	16	35	43

What can be immediately seen from the Charles River Data Base in comparison to the cyanazine study is that the cyanazine (Table II) adenocarcinoma control (8%) is within the range of 0 to 16 percent for adenocarcinoma and that the adenocarcinoma incidences from (Table II) for 5, 25, and 50 ppm (20, 30, and 24%, respectively) exceed the range of historical control from Charles River. Other interesting features is that the CR mean for adenocarcinoma is 7.4 percent (cyanazine control was 8.0%) and that is 3 of 11 studies, the CR historical control for adenocarcinoma was 0 percent.

Discussion:

Cyanazine is unequivocally a mammary gland carcinogen at doses of 5, 25, and 50 ppm. The registrant is required to provide the statistically appropriate historical control data for adenocarcinomas and malignant mammary gland carcinomas in order to compare with the results of the cyanazine study.

Additionally, historical control data for non-neoplastic lesions has been requested.

The Biostatistics Team of SACB needs to perform the usual statistical analysis on the "pulled" data for the Peer Review.

With respect to a NOEL (including carcinogenicity) for chronic toxicity, the 1.0 ppm level was without apparent toxic effect. At the LEL of 5.0 ppm, mammary gland adenocarcinomas occurred at significant incidences.

With respect to a second NOEL (excluding carcinogenicity), the 5.0 ppm level is the NOEL and the LEL is 25 ppm with mammary gland masses (gross necropsy observations), toxic signs in males (hyperactivity), and decreased body weight gain in females (statistically significant and reaching 11%) were observed.

Attachments

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ATTACHMENTS

Benign and/or Malignant Mammary Gland Tumors in Tumor-Bearing Female Crl:CD BR (Sprague-Dawley) Rats in the 2-Year Rat Feeding Study with Cyanazine

Dose (ppm)	Animal No.	Days on Study	Tumor Type	Degree of Hyperplasia
0	422283	486 (FD)	Fibroadenoma	
0	422285	697 (SE)	Fibroadenoma	
0	422288	663 (SE)	Fibroadenoma, multiple	
0	422291	606 (FD)	Adenocarcinoma, multiple	
0	422292	734 (TK)	Fibroadenoma	Mild
0	422293	693 (SE)	Fibroadenoma	
0	422294	734 (TK)	Fibroadenoma	
0	422296	734 (TK)	Fibroadenoma	
0	422297	721 (SE)	Multiple Adenoma, Multiple Fibroadenoma	
0	422299	734 (TK)	Fibroadenoma	
0	422300	734 (SE)	Fibroma	Mild Hyperplasia
0	422302	679 (SE)	Fibroadenoma	
0	422308	608 (SE)	Multiple Adenocarcinoma	Severe
0	422310	735 (TK)	Adenocarcinoma, Fibroadenoma, multiple	Severe
0	422323	735 (TK)	Fibroadenoma	
0	422325	735 (TK)	Multiple, Fibroadenoma	
0	422328	335 (FD)	Adenocarcinoma	Minimal
0	422329	621 (FD)	Fibroadenoma	Mild
0	422330	620 (FD)	Multiple, Fibroadenoma	Mild
0	422331	582 (FD)	Multiple, Fibroadenoma	Mild
0	422332	735 (TK)	Adenoma, Fibroadenoma	Mild
0	422333	693 (SE)	Fibroadenoma	
0	422335	691 (FD)	Fibroadenoma	
0	422336	735 (TK)	Fibroadenoma, Adenocarcinoma	
0	422341	613 (SE)	Fibroadenoma	Severe
0	422344	681 (FD)	Fibroadenoma	
1.0	422345	734 (TK)	Adenoma	Severe
1.0	422346	734 (TK)	Fibroadenoma	Mild
1.0	422347	734 (TK)	Adenoma	
1.0	422348	585 (SE)	Fibroadenoma, multiple	
1.0	422349	654 (FD)	Fibroadenoma	Mild
1.0	422351	734 (TK)	Adenoma, Adenocarcinoma	Moderate
1.0	422355	430 (FD)	Fibroadenoma	Mild
1.0	422360	494 (FD)	Adenoma	Mild
1.0	422362	735 (TK)	Fibroadenoma, multiple	
1.0	422364	685 (FD)	Adenoma, Adenocarcinoma, multiple	
1.0	422368	603 (FD)	Fibroadenoma	Mild
1.0	422370	735 (TK)	Fibroadenoma, multiple	
1.0	422373	735 (TK)	Fibroadenoma	
1.0	422374	693 (SE)	Fibroadenoma	Minimal
1.0	422377	735 (TK)	Fibroadenoma	
1.0	422380	735 (TK)	Fibroadenoma	

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Dose (ppm)	Animal No.	Days on Study	Tumor Type	Degree of Hyperplasia
1.0	422381	625 (FD)	Fibroadenoma	
1.0	422384	530 (FD)	Fibroadenoma	Mild
1.0	422385	693 (FD)	Fibroadenoma	
1.0	422386	735 (TK)	Fibroadenoma	
1.0	422387	736 (TK)	Adenocarcinoma, multiple	
1.0	422389	736 (TK)	Fibroadenoma	
1.0	422390	494 (FD)	Fibroadenoma	Moderate
1.0	422393	736 (TK)	Fibroadenoma, multiple	
1.0	422398	614 (FD)	Fibroadenoma	
1.0	422399	704 (FD)	Fibroadenoma, multiple	Mild
1.0	422400	736 (TK)	Adenocarcinoma	
1.0	422402	369 (SD)	Adenoma, multiple adenocarcinoma	
1.0	422403	736 (TK)	Adenocarcinoma	Moderate
1.0	422406	553 (SE)	Adenocarcinoma	Moderate
5.0	422409	728 (SE)	Fibroadenoma, multiple	Mild
5.0	422414	734 (TK)	Fibrosarcoma	
5.0	422416	734 (TK)	Adenocarcinoma, multiple	
5.0	422419	735 (TK)	Adenocarcinoma	
5.0	422423	603 (SE)	Fibroadenoma	
5.0	422425	497 (SE)	Adenocarcinoma	Mild
5.0	422427	735 (TK)	Fibroma	
5.0	422428	601 (FD)	Adenoma	Severe
5.0	422431	735 (TK)	Adenocarcinoma, multiple fibroadenoma	Severe
5.0	422435	571 (SE)	Fibroadenoma	Mild
5.0	422436	369 (SD)	Fibroadenoma	
5.0	422439	566 (FD)	Adenoma	Moderate
5.0	422440	540 (FD)	Fibroadenoma	Mild
5.0	422441	735 (TK)	Fibroadenoma, multiple	
5.0	422442	735 (TK)	Fibroadenoma	
5.0	422443	697 (FD)	Adenocarcinoma, multiple	
5.0	422444	671 (FD)	Fibroadenoma	
5.0	422447	679 (FD)	Fibroadenoma, adenocarcinoma	
5.0	422448	735 (TK)	Fibroadenoma	
5.0	422449	693 (SE)	Adenocarcinoma, multiple	Moderate
5.0	422451	735 (TK)	Fibroadenoma	
5.0	422452	655 (FD)	Adenocarcinoma, multiple	Moderate
5.0	422453	736 (TK)	Fibroadenoma, multiple	
5.0	422454	693 (SE)	Fibroadenoma, multiple	
5.0	422457	494 (FD)	Adenoma, Fibroadenoma Adenocarcinoma	Mild
5.0	422458	550 (FD)	Fibroadenoma	
5.0	422459	727 (FD)	Fibroadenoma, Adenocarcinoma, multiple	Moderate
5.0	422461	736 (TK)	Fibroadenoma	
5.0	422463	736 (TK)	Fibroadenoma, multiple adenocarcinoma	
5.0	422464	678 (SE)	Adenocarcinoma	

Dose (ppm)	Animal No.	Days on Study	Tumor Type	Degree of Hyperplasia
5.0	422466	665 (FD)	Fibroadenoma, multiple	
5.0	422468	736 (TK)	Fibroadenoma, multiple	
25.0	422474	615 (SE)	Adenocarcinoma	Moderate
25.0	422475	715 (FD)	Fibroadenoma, multiple	
25.0	422476	648 (FD)	Adenoma	
			Fibroadenoma, multiple	Moderate
25.0	422478	369 (SD)	Adenocarcinoma	Mild
25.0	422484	718 (FD)	Fibroadenoma	
25.0	422485	735 (TK)	Adenoma, adenocarcinoma	
25.0	422486	735 (TK)	Fibroadenoma	
25.0	422489	631 (FD)	Fibroadenoma, multiple	Moderate
25.0	422491	735 (TK)	Fibroadenoma, adenocarcinoma	
25.0	422493	735 (TK)	Fibroadenoma, adenocarcinoma	
25.0	422494	735 (TK)	Fibroadenoma, adenocarcinoma	
25.0	422496	677 (FD)	Fibroadenoma	
25.0	422497	623 (FD)	Adenoma, fibroadenoma, multiple Adenocarcinoma	
25.0	422498	735 (TK)	Fibroadenoma, adenocarcinoma, multiple	Mild
25.0	422501	735 (TK)	Fibroadenoma, multiple	
25.0	422502	462 (SE)	Carcinosarcoma	Mild
25.0	422507	735 (TK)	Adenocarcinoma	
25.0	422508	704 (FD)	Adenocarcinoma	
25.0	422510	683 (FD)	Adenoma, fibroadenoma, adenocarcinoma, multiple	Mild
25.0	422511	735 (TK)	Adenocarcinoma, multiple	
25.0	422512	369 (SD)	Adenocarcinoma	Mild
25.0	422513	736 (TK)	Adenocarcinoma, multiple	
25.0	422516	496 (FD)	Fibroadenoma, adenocarcinoma	
25.0	422520	736 (TK)	Adenocarcinoma, multiple	
25.0	422522	736 (TK)	Fibroadenoma	
25.0	422523	663 (FD)	Fibroadenoma, adenocarcinoma	
25.0	422524	489 (FD)	Adenocarcinoma	
25.0	422526	714 (FD)	Fibroadenoma	
25.0	422527	736 (TK)	Adenocarcinoma	
25.0	422530	736 (TK)	Fibroadenoma	
50.0	422533	634 (SE)	Fibroadenoma	
50.0	422536	539 (SE)	Adenocarcinoma	Mild
50.0	422537	686 (SE)	Fibroadenoma	
50.0	422538	456 (FD)	Fibroadenoma	
50.0	422539	720 (FD)	Fibroadenoma, multiple	Severe
50.0	422541	734 (TK)	Adenocarcinoma, multiple	Mild
50.0	422542	369 (SD)	Fibroadenoma	
50.0	422543	655 (SE)	Fibroadenoma	
50.0	422544	735 (TK)	Adenoma, multiple, adenocarcinoma, multiple	Moderate
50.0	422545	735 (TK)	Adenocarcinoma	
50.0	422546	693 (SE)	Fibroadenoma, multiple	
50.0	422548	735 (TK)	Fibroadenoma	Mild

Dose (ppm)	Animal No.	Days on Study	Tumor Type	Degree of Hyperplasia
50.0	422549	654 (SE)	Fibroadenoma	
50.0	422551	648 (SE)	Fibroadenoma	
50.0	422558	535 (SE)	Adenocarcinoma	Moderate
50.0	422560	668 (SE)	Fibroadenoma	
50.0	422561	735 (TK)	Fibroadenoma	Moderate
50.0	422562	729 (SE)	Fibroadenoma	Mild
50.0	422563	663 (FD)	Fibroadenoma, multiple	
50.0	422567	735 (TK)	Adenocarcinoma, multiple	Mild
50.0	422568	735 (TK)	Fibroadenoma, multiple Fibrosarcoma	
50.0	422569	735 (TK)	Fibroadenoma	
50.0	422570	735 (TK)	Adenocarcinoma, multiple Fibrosarcoma	
50.0	422572	534 (FD)	Fibroadenoma	Moderate
50.0	422573	736 (TK)	Fibroadenoma Adenocarcinoma, multiple	
50.0	422575	736 (TK)	Fibroadenoma	Severe
50.0	422576	554 (FD)	Fibroadenoma, multiple Adenocarcinoma, multiple	Moderate
50.0	422577	736 (TK)	Adenoma	
50.0	422579	464 (FD)	Adenocarcinoma, multiple	Moderate
50.0	422581	557 (FD)	Fibroadenoma, multiple	
50.0	422586	736 (TK)	Adenocarcinoma	
50.0	422588	418 (FD)	Fibroadenoma Adenocarcinoma	Mild
50.0	422589	728 (FD)	Adenocarcinoma	Mild
50.0	422590	736 (TK)	Fibroadenoma, multiple Adenocarcinoma	
50.0	422591	559 (FD)	Fibroadenoma	
50.0	422592	736 (TK)	Fibroadenoma Adenocarcinoma	Mild

Benign and Malignant Palpable Masses
Observed Clinically During the Study

Dose	Animal Number	Cause of Death	Mammary Gland Masses	Day First Observed
0	422283	--	Rt. mass & Lt. Mass	343, 357
0	422285	PA	Mass 1	651
0	422288	FA	Multiple	343
0	422291	AC	Multiple	371
0	422292	TK	Mass 1	707
0	422293	PC	Mass 1 (neck)	693
0	422294	TK	Mass 1	399
0	422296	TK	Mass 1, Mass 2	581, 595
0	422297	PA	Multiple	413
0	422299	TK	Mass 1	707
0	422300	PA	Mass 1, Mass 2	521, 585
0	422302	PA	Mass 1	679
0	422308	PA	Multiple	343, 343
0	422310	TK	Multiple	497, 707
0	422323	TK	Mass 1	623
0	422325	TK	Multiple	413
0	422328	AC	Mass 1	203
0	422329	PA	Mass 1, 2	301
0	422330	FA	Mass 1, 2	385, 595
0	422331	--	Mass 1	385
0	422332	TK	No gross clinical lesions	735
0	422333	FA	Mass 1	539
0	422335	TK	Mass 1, 2	231, 609
0	422336	TK	Mass 1, 2	525, 665
0	422341	PA	Mass 1, 2	511, 581
0	422344	--	Mass 1	441
1.0	422345	TK	Mass 1	455
1.0	422346	TK	Mass 1, 2, 3, 4	679, 707
1.0	422347	TK	Data not reported	Data not reported
1.0	422348	PA	Mass 1, 2	455, 455
1.0	422349	--	Mass 1, 2	553, 637
1.0	422351	TK	Mass 1	595
1.0	422355	PA	Mass 1	385
1.0	422360	PA	Mass 1, 2	301, 427
1.0	422362	TK	Mass 1	735
1.0	422364	Uterine tumor	Mass 1-5	301-581
1.0	422368	FA	Mass 1	455
1.0	422370	TK	Mass 1-4	567-665
1.0	422373	TK	No clinically observable mass	
1.0	422374	FA	Mass 1	413
1.0	422377	TK	Mass 1	441
1.0	422380	TK	Mass 1	735
1.0	422381	PA	No clinically observed masses	
1.0	422384	PC	Mass 1	343

Dose	Animal Number	Cause of Death	Mammary Gland Masses	Day First Observed
1.0	422385	PA	No clinically observed masses	
1.0	422386	TK	No clinically observed masses	
1.0	422387	TK	Mass 1	343
1.0	422389	TK	Mass 1	567
1.0	422390	PA	No clinically observed masses	
1.0	422393	TK	Mass 1	623
1.0	422398	PA	No clinically observed masses	
1.0	422399	--	Mass 1, 2	511, 553
1.0	422400	TK	Mass 1	707
1.0	422402	SD (sacrificed by design?)	No clinically observed masses	
1.0	422403	TK	Mass 1	497
1.0	422406	PA	Mass 1, 2, 3	315, 329, 413
5.0	422409	PA	Masses 1-9	413-511
5.0	422414	TK	Mass 1	343
5.0	422416	TK	Mass 1	595
5.0	422419	TK	Mass 1	441
5.0	422423	PA	Mass 1	567
5.0	422425	AC	Mass 1	357
5.0	422427	TK	Mass 1	567
5.0	422428	Undetermined	Masses 1-4	301-553
5.0	422431	TK	Mass 1, 2	595, 735
5.0	422435	--	Mass 1, 2	329, 343
5.0	422436	Interim Kill (day 369)	Mass 1	343
5.0	422439	PA	Mass 1	357
5.0	422440	--	Mass 1	399
5.0	422441	TK	Masses 1-3	553, 595, 679
5.0	422442	TK	Mass 1	511
5.0	422443	PA	Mass 1	245
5.0	422444	PC	Mass 1, 2	343, 343
5.0	422447	AC	Mass 1	637
5.0	422448	TK	Mass 1	665
5.0	422449	PA	Masses 1-4	567-651
5.0	422451	TK	Mass 1	623
5.0	422452	AC	Masses 1-6	217-497
5.0	422453	TK	Mass 1	539
5.0	422454	Fibrosarcoma (peritoneum)	Mass 1 [b]	637
5.0	422457	AC	Masses 1-3	301-455
5.0	422458	PA	Masses 1-4	329-343
5.0	422459	PA	Mass 1, 2	665, 721
5.0	422461	TK	Mass 1, 2	357, 539
5.0	422463	TK	Mass 1-3	539, 637, 651
5.0	422464	PA	Mass 1	595
5.0	422466	PA	Mass 1, 2	553, 581
5.0	422468	TK	No clinically observed masses	
25.0	422474	PC	Mass 1	315
25.0	422475	PA	Masses 1-4	329-357

Dose	Animal Number	Cause of Death	Mammary Gland Masses	Day First Observed
25.0	422476	PA	Masses 1, 2, 3	357, 371, 371
25.0	422478	SD	Mass 1, 2	315, 315
25.0	422484	PA	Masses 1-5	525-707
25.0	422485	TK	Mass 1, 2	665, 707
25.0	422486	TK	Mass 1	567
25.0	422489	FA	Mass 1, 2	161, 511
25.0	422491	TK	Mass 1, 2	301, 567
25.0	422493	TK	Mass 1, 2	581, 595
25.0	422494	TK	Mass 1, 2, 3	413, 413, 413
25.0	422496	Uterine tumor	Mass 1, 2, 3	385, 385, 385
25.0	422497	AC	Mass 1, 2, 3	413, 595, 609
25.0	422498	TK	Masses 1-5	385-637
25.0	422501	TK	No clinically observed masses	
25.0	422502	Carcino-sarcoma	Mass 1	167
25.0	422507	PA	Mass 1	203
25.0	422510	Fibrosarcoma (lung)	Mass 1, 2, 3	287-603
25.0	422511	TK	Mass 1, 2, 3	315, 581, 721
25.0	422512	SD	Mass 1	231
25.0	422513	TK	Mass 1	539
25.0	422516	PA	Mass 1, 2, 3	273-287
25.0	422520	TK	Mass 1, 2	441, 441
25.0	422523	PA	Mass 1, 2	315, 469
25.0	422524	AC	Clinical data not reported	
25.0	422526	PA	Mass 1, 2	399, 413
25.0	422527	TK	No observable clinical masses	
25.0	422530	TK	Mass 1, 2, 3	399, 422, 455
50.0	422533	PA	No observable clinical masses	
50.0	422536	AC	Mass 1	413
50.0	422537	FA	Masses 1-4	413-686
50.0	422538	PA	Mass 1	259
50.0	422539	PA	Mass 1, 2, 3	371, 371, 693
50.0	422541	TK	Mass 1	399
50.0	422542	SD	Mass 1	217
50.0	422543	FA	Mass 1	343
50.0	422544	TK	Mass 1	665
50.0	422545	TK	Mass 1	581
50.0	422546	PA	Mass 1	623
50.0	422548	TK	Mass 1	369
50.0	422549	PA	Mass 1	329
50.0	422551	PC	Mass 1, 2	287, 371
50.0	422558	PA	Mass 1	441
50.0	422560	FA	Mass 1, 2, 3	595, 581, 609
50.0	422561	TK	No clinically observed masses	
50.0	422562	PA	Mass 1	343
50.0	422563	FA	Mass 1, 2, 3	315, 371, 595
50.0	422567	TK	Mass 1, 2	217, 483
50.0	422568	TK	Mass 1, 2, 3	167, 651, 735

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Dose	Animal Number	Cause of Death	Mammary Gland Masses	Day First Observed
50.0	422569	TK	No clinically observed masses	
50.0	422570	TK	Mass 1, 2, 3	483, 651, 707
50.0	422572	FA	Mass 1, 2	343, 343
50.0	422573	TK	Masses 1-5	119-441
50.0	422575	TK	Mass 1, 2	539, 553
50.0	422576	AC	Mass 1, 2, 3	301, 497, 511
50.0	422577	TK	Mass 1, 2	273, 609
50.0	422579	PA	Mass 1, 2	343, 441
50.0	422581	PA	Mass 1, 2	273, 343
50.0	422586	TK	Mass 1, 2, 3	287, 301, 315
50.0	422588	PA	Mass 1, 2	245, 245
50.0	422589	PA	Mass 1	315
50.0	422590	TK	Masses 1 - 5	287-315
50.0	422591	--	Mass 1, 2	511, 539
50.0	422592	TK	Mass 1	651

AC = Mammary adenocarcinoma
PA = Pituitary adenoma
FA = Mammary Fibroadenoma
TK = Terminal Kill
PC = Pituitary carcinoma
SD = Sacrificed by design
--- = Cause of death unrelated to tumor of concern.

Bladex

Page_____ is not included in this copy.

Pages 68 through 74 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

____ FIFRA registration data.

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Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head
Section I, Toxicology Branch I - IRS (H7509C)

Roger Gardner 2/11/91

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DATA EVALUATION REPORT

Study Type: Mouse oncogenicity - 83-2 TOX Chem No.: 188C

Accession Number: 247295-298 MRID No.: N/A

Test Material: Cyanazine, 96.4% purity

Synonyms: Bladex

Study Number: 1493

Sponsor: Shell Chemical Company

Testing Facility: Shell Toxicology Lab (Tunstall)

Title of Report: A Two-Year Feeding Study of Bladex in Mice.

Author: J.B.M. Gellatly

Report Issued: December 1981

Conclusions:

This review supplements the HED review of May 24, 1982 by W. Dykstra.

The oncogenic potential was negative up to 1000 ppm (HDT), which exceeded the MTD.

The MTD was 250 ppm. At this level, there were significant (10-23%) decreases in body weight gain ranging up to 14 percent in males and 23 percent in females during the entire study. Part of the decreased body weight gain was due to decreased food consumption, although the remainder reflects the direct toxicity of cyanazine.

At 1000 ppm, palatability problems were seen as significant excess food spillage by both sexes during the entire study.

The NOEL for clinical signs, gross necropsy findings, increased incidences of histological effects, and clinical pathology results was 25 ppm.

The NOEL for decreased relative kidney weight to body weight was 10 ppm.

The NOEL for systemic toxicity may be 10 ppm (LDT), although 3 to 7 percent body weight gain decreases were observed in females during most of the study.

The incidence of hemangiosarcoma of the spleen in males was 1/100 (1%), 4/50 (8%)*, 2/50 (4%), 0/50, and 0/50 for the 0, 10, 25, 250, and 1000 ppm groups, respectively. The incidence of total number of tumor-bearing male mice with hemangiosarcomas was 3, 12*, 4, 2, and 2 percent for the 0, 10, 25, 250, and 10,000 ppm groups, respectively (*$p < 0.05$) (see Table 1).

The lack of dose-response, the occurrence of a historical range for CD-1 male mice in the open literature up to 13.3 percent, and the lack of increase in this tumor type in treated females (control females had 2/100) resulted in the conclusion that this tumor type was not compound-related at the 10 ppm level, although it was statistically significant ($p < 0.05$).

Classification: Core-Minimum

Special Review Criteria (40 CFR 154.7): N/A

A. Materials:

1. Test Compound - Cyanazine technical (WL 19805); Description: Broad-spectrum herbicide; Batch No. 8-21-0-0; Purity: 96.4 percent; Contaminants: List in CBI Appendix.

2. Test Animals - Species: Mouse; Strain: CD(SPF); Age: 35 days; Weight: Not given; Source: Shell Toxicology Laboratory.

B. Study Design:

1. Animal Assignment - Animals were assigned randomly to the following test groups (no interim sacrifice):

Test Group	Dose in Diet (ppm)	Main Study 24 Months Male	Female
Control	0	100	100
Low (LDT)	10	50	50
Mid (MDT)	25	50	50
Mid (MDT)	250	50	50
High (HDT)	1000	50	50

2. Diet Preparation - Diet was prepared monthly and stored at room temperature. Samples of treated food were analyzed for stability and concentration at monthly intervals.

Results - Analyses of diet for stability and concentration for cyanazine were within ± 10 percent of nominal concentrations during the 2-year period. The average diet analyses for concentrations over the 2-year period were 10.0 ± 4.5, 24.8 ± 4.3, 240 ± 5.2, and 983 ± 5.5 ppm (± is coefficient of variation). Stability analysis at 0, 14, 21, and 28 days were within 10 percent of nominal values, and showed that cyanazine was stable in the diet up to 28 days.

3. Animals received food (Laboratory Animal Diet #1[a] and 2[b]) obtained from Spratt's Patent, Ltd. and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: $p < 0.05$ or 0.01 were significant.

5. Quality assurance was performed and signed by J.B.M. Gellatly.

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[a] First week.
[b] Remainder of study.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality. No compound-related clinical signs were observed in male mice. In female mice, 56 percent of females at 1000 ppm showed poor condition compared with 26 percent females in controls. The incidence of skin sores/fur loss was 20 percent in both the 250 and 1000 ppm female groups in comparison to 10 percent in controls. The NOEL is 25 ppm for this finding. There were no other compound-related clinical signs. The NOEL for clinical signs is 25 ppm.

Results - Toxicity

Mortality (survival) - The following table shows percentage survival after 2 years on study. There were no compound-related effects on survival in treated males and a slight decrease in the 250 and 1000 females in comparison to controls which was not statistically significant.

Survival of Male and Female Mice Exposed to BLADEX for 2 Years

Treatment (ppm)	Percent Survival Males	Females
0	54	49
10	46	48
25	56	50
250	54	38*
1000	58	42

*Animal number 278 female was fed control diet from week 80 and has therefore been excluded from all tables and statistical analyses.

2. Body Weight - Animals were weighed weekly for 13 weeks, then monthly for the remainder of the study.

Results - Statistically significant ($p < 0.01$) decreases in body weight gain (10 to 32% from weeks 1 to 105) were observed in males and females exposed to dietary levels of 25 (females only), 250, and 1000 ppm throughout the 104-week study. At 25 ppm in males, significant decreases were observed at weeks 11, 13, 16, 20, 36, 40, 44, 52 to 76, and at weeks 88, 92, and 104. At 10 ppm, males showed significant decreases at weeks 44, 60, 72, 90, and 104 (decreases of about 3%). Females at 10 ppm showed

significant body weight gain decreases from week 10 onward to week 104. The decreases in weight gain ranged from 3 to 7 percent. These marginal (less than 10%) effects in body weight gain at 10 ppm in both sexes are sufficiently small to perhaps consider 10 ppm as the NOEL for body weight in the study. The table of body weight data below shows the terminal differences between groups.

Body Weight Data

	Week Number	Treatment (ppm) 0	10	25	250	1000	Standard Deviation of a Single Observation
		Group Size (N)					
Males	N	54	23	28	27	29	
	105+	48.1	45.1	46.6	42.3**	36.3**	4.64
Females	N	49	24	25	19	21	
	105+	41.6	36.8*	40.0*	34.1**	28.5**	5.06

*Animal number 278 female was fed control diet from week 80 and has therefore been excluded from all tables and statistical analyses.
+ = Adjusted for initial body weight.
*$p \leq 0.05$. Significance of difference between treatment and control means.
**$p \leq 0.01$. Significance of difference between treatment and control means.

3. Food Consumption and Compound Intake - Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results - Food Consumption

Food Efficiency: Compound Intake - A reduction in palatability at the high dose was evidenced as increased food spillage. Food spillage was higher than control in males and females at 1000 ppm.

Statistically significant decreases in food intake by males were observed at 1000 ppm (weeks 1, 3-12, 16, 20, 28-60, 68, 80, 84, and 104), and at 250 ppm (weeks 1, 3-20, 28-60, 68, 80, 84, and 104). At 25 ppm, significant reductions throughout the study were observed and no significant reductions were observed at 10 ppm in males.

Statistically significant decreases in food intake by females were observed at 1000 ppm (weeks 1-7, 36-40, 48-60,

68, 72, 80, and 88-105), at 250 ppm (weeks 1, 3-7, 36, 40, 48-60, 72, 80, and 88-100). At 25 ppm, significant reductions were seen (weeks 3-7, 36, 40, 48, and 60). Food intake at 10 ppm was comparable to controls for most of the study.

The overall food conversion efficiency (FCE) was statistically significantly reduced for males and females at 250 and 1000 ppm for the duration of the study.

4. Ophthalmological examinations were not performed.

5. Blood was collected at 24 months for hematology and clinical analysis from all surviving animals. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*		Total plasma protein (TP)
X	Hemoglobin (HGB)*	X	Leukocyte differential count
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB (MCH)
X	Erythrocyte count (RBC)*	X	Mean corpuscular HGB concentration (MCHC)
	Platelet count*	X	Mean corpuscular volume (MCV)

Results - Statistically significant depressions were seen in high-dose female mice in hemoglobin (13.30 [control] vs. 12.53 g/100 mL [high-dose], mean corpuscular hemoglobin [17.81 vs. 17.12 pg], and mean corpuscular hemoglobin concentration [33.57 vs. 32.02 g/100 mL]). Evaluation of the prepared blood films of males and females showed at the high-dose a decrease in the percentage of lymphocytes in both sexes. In females, there was an increase in percentage of monocytes and eosinophils at 250 ppm and an increase in percentage of neutrophils at 1000 ppm.

In males, there was an increase in the percentage of monocytes and a decrease in the absolute number of neutrophils at 250 ppm. There were no significant differences between the total leukocyte counts of treated groups in comparison to controls for both sexes.

The NOEL for hematological findings is 25 ppm.

b. Clinical Chemistry

X		X	
	Electrolytes:		Other:
	Calcium*		Albumin*
	Chloride*		Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
	Phosphorus*		Cholesterol*
	Potassium*		Globulins
	Sodium*	X	Glucose*
	Enzymes		Total Bilirubin*
X	Alkaline phosphatase	X	Total Protein*
	Cholinesterase		Triglycerides
	Creatinine phosphokinase*		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
X	Protein electrophoresis		

Results - Female mice at the high-dose showed a statistically significant decrease in glucose (6.16 vs. 5.66 mmol/L (control vs. high-dose) and an increase in total protein (55.6 vs. 62.1 g/L).

Fractionation of the proteins by electrophoresis showed a decrease in albumin and increase in the beta-globulin fraction in females at the high dose.

In high-dose males, there was an increase in the alpha-1-globulin fraction. There were no other compound-related clinical chemistry findings. The NOEL for clinical chemistry is 250 ppm.

6. Urinalysis - Urine was not collected from fasted animals.

7. Sacrifice and Pathology - All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X		X		X	
	Digestive System		Cardiovasc./Hemat.		Neurologic
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus	X	Bone marrow*	X	Spinal cord (3 levels)
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (optic nerve)
X	Jejunum*	X	Thymus*		Glandular
X	Ileum*		Urogenital	X	Adrenals*
X	Cecum*	XX	Kidneys*		
X	Colon*	X	Urinary bladder*		

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Rectum*	XX	Testes*	X	Lacrimal gland
XX	Liver*	X	Epididymides	X	Mammary gland*
X	Gallbladder*	X	Prostate	X	Parathyroids*
X	Pancreas*	X	Seminal vesicle	X	Thyroids*
	Respiratory	X	Ovaries		Other
X	Trachea*	X	Uterus*	X	Bone*
X	Lung*			X	Skeletal muscle*
				X	Skin
				X	All gross lesions and masses

Results

a. Organ Weight - Numerical values for unadjusted and adjusted (terminal body weight) are attached to the report.

The following table, presented in the report, shows the differences among groups. The NOEL for relative organ weights/body weight is 10 ppm and the LEL is 25 ppm. At the LEL, there were (adjusted for body weight) in males decreased relative kidney weights. In females the NOEL is 25 ppm and at the LEL of 250 ppm there are increased relative brain weights (this appeared in males at 250 ppm, also). Additionally, at 250 ppm there were decreased relative heart and relative kidney weights in males at 250 ppm.

Table 6.4. Summary of Statistically Significant Differences in Unadjusted, Adjusted (Terminal Body Weight) and Relative Organ Weights - 2-Year Feeding Study of 0.0 to 1000 ppm BLADEX

Dietary Concentration (ppm)	Males					Females				
	0	10	25	250	1000	0	10	25	250	1000
Organs										
Unadjusted										
Brain					D				D	D
Heart			D	D	D					D
Liver					D					D
Testes										
Kidneys		D	D	D	D				D	D

D - Decrease of statistical significance.

Table 6.4. Summary of Statistically Significant Differences in Unadjusted, Adjusted (Terminal Body Weight) and Relative Organ Weights - 2-Year Feeding Study of 0.0 to 1000 ppm BLADEX (cont'd)

Dietary Concentration (ppm)	Males					Females				
	0	10	25	250	1000	0	10	25	250	1000
Adjusted (To Terminal Body Weight)										
Brain				D	D	NCR	NCR	NCR	NCR	NCR
Heart	NCR	NCR	NCR	NCR	NCR					
Liver										
Testes			D	D	D					D
Kidneys										
Adjusted (To Terminal Body Weight)										
Brain				I	I				I	I
Heart					I					I
Liver										
Testes					I					
Kidneys										I
Terminal Body Weight				D	D		D	D	D	D

D - Decrease of statistical significance.
I - Increase of statistical significance.
NCR - No significant change in relationship.

b. Gross Pathology - There was one compound-related gross pathologic lesion in female mice (Tables 6.7 [page 100]), 6.8, 6.9, 6.10, 6.11, 6.12, 6.13, and 6.14 [male mice]; 6.15, 9.16, 6.17, 6.18, 6.19, 6.20, 6.21 [female mice]).

[redacted]ales had an increased incidence of ulcerated skin [redacted]erved at necropsy in the 250 and 1000 ppm groups [redacted] comparison to controls. The incidence of this grossly observed lesion in decedents was 3/51 (6%) at 0 ppm, 5/30 (17%) at 250 ppm, and 5/29 (17%) at 1000 ppm. There were no findings of this type at 25 ppm.

c. Microscopic Pathology

1) Non-Neoplastic - The following organs had increased incidences of non-neoplastic histopathological lesions.

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Liver

	Males					Females				
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
No. Examined	100	50	50	50	50	100	50	50	49	50
Centrilobular Parenchymal Hypertrophy (Percent)	12 12%	NOEL = 250 ppm LEL = 1000 ppm 2 4%	5 10%	6 12%	10 20%					
Parenchyma, Atrophy (Percent)						39 39%	18 36%	17 34%	28 57%	34 68%

NOEL = 25 ppm
LEL = 250 ppm

These liver lesions can be considered cyanazine-related lesions although atrophy is associated with poor nutrition (females) whereas cellular enzyme induction and/or toxicity (males) is associated with hypertrophy.

Kidney

	Males					Females				
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
No. Examined	100	50	50	50	50	100	50	50	49	50
Diffuse Cortical Tubular dilation (Percent)	10 10%	NOEL = 25 ppm LEL = 250 ppm 2 4%	2 4%	10 20%	15 30%					
Diffuse Cortical Epithelium Vacuolation (Percent)						5 5%	2 4%	1 2%	5 10%	18 36%

NOEL = 25 ppm
LEL = 250 ppm

These two kidney lesions are considered due directly to the toxic effect of cyanazine on the kidney. Both dilation of cortical tubules and vacuolation of cortical epithelium are serious toxic effects.

Heart

	Males					Females				
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
No. Examined	100	50	50	50	50	100	50	50	49	50
Acute Subacute Myocarditis	NOEL = 250 ppm LEL = 1000 ppm									
	3	1	3	1	9					
(Percent)	3%	2%	6%	2%	18%					
Basal myocardial fibrosis	20	10	11	6	15					
(Percent)	20%	20%	22%	12%	30%					
Basal myocardial fibrosis						14	6	3	11	22
(Percent)						14%	12%	6%	22%	44%
Nonbasal myocardial fibrosis						2	2	1	5	14
(Percent)						2%	4%	2%	10%	28%

NOEL = 25 ppm
LEL = 250 ppm

These heart lesions in males and females may reflect the poor nutritional status of the mice at 250 and 1000 ppm rather than direct toxic effects of cyanazine to myocardial tissue. However, it should be noted that myocardial effects in mice occurred with propazine and similar effects occurred in dogs with atrazine.

Adrenals

	Females				
Dose (ppm)	0	10	25	250	1000
No. Examined	100	49	50	49	50
Cortical lipid depletion	1	3	2	7	9
(Percent)	1%	6%	4%	14%	18%

NOEL = 25 ppm
LEL = 250 ppm

The lipid depletion of the adrenals most likely reflects the poor nutritional status of the 250 and 1000 ppm groups.

Brain

Females

Dose (ppm)	0	10	25	250	1000
No. Examined	100	50	50	50	50
Corpora calcification of brain stem	27	11	12	18	14
(Percent)	27%	22%	24%	36%	28%

Although there is an increased percentage at 250 ppm, the lack of dose response at 1000 ppm leads to the conclusion that the finding at 250 ppm is not compound-related.

Skin Subcutis

Females

Dose (ppm)	0	10	25	250	1000
No. Examined	96	48	47	49	49
Skin, patchy ulceration	3	0	0	5	6
(Percent)	3%	0%	0%	10%	12%

NOEL = 25 ppm
LEL = 250 ppm

These histopathological lesions are directly due to cyanazine and can be correlated with the gross macroscopic findings and clinical signs of females in these groups.

Bone Marrow

	Males					Females				
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
No. Examined	94	48	49	47	49	97	48	48	48	49
Prominent hematopoiesis	18	13	10	6	27	21	12	12	19	26
(Percent)	18%	26%	20%	12%	54%	21%	24%	24%	38%	52%
		NOEL = 250 ppm LEL = 1000 ppm					NOEL = 25 ppm LEL = 250 ppm			

The increase in hematopoiesis in the bone marrow of both sexes at 250 and 1000 ppm most probably reflects the frequently seen compensatory response of this tissue, which in this case is due to the poor nutritional status of these groups.

The overall NOEL for non-neoplastic lesions is 25 ppm for both sexes in this study.

2) Neoplastic

Males and Females - Tables 6.64 through 6.68 - There was an increased incidence in males of hemangiosarcoma of the spleen at 10 and 25 ppm which was statistically significant at 10 ppm ($p < 0.05$). This is shown in Table 6.63 as presented below:

Incidence of Tumors

	Males					Females				
Tumors / Dietary Conc. (ppm) / Number of Animals Examined	0 100	10 50	25 50	250 50	1000 50	0 100	10 50	25 50	250 49	1000 50
Lymphoreticular Tissues										
Lymphoblastic lymphosarcoma	5	1	1	2	3	9	5	3	6	4
Reticulum cell sarcoma	2	2	1	2	3	3	4	4	3	3
Stem cell leucemia	1	1		1	1	1			2	1
Myeloid leucemia	1	1	1	1		1	1			
Erythroblastic sarcoma				1		1				

Incidence of Tumors (cont'd)

Tumors	Dietary Conc. (ppm) / Number of Animals Examined	Males 0	Males 10	Males 25	Males 250	Males 1000	Females 0	Females 10	Females 25	Females 250	Females 1000
		100	50	50	50	50	100	50	50	49	50
Spleen - Hemangiosarcoma		1	4*	2			1				
Percentages		1%	*8%	4%	0	0	1%	0	0	0	0
Spleen - Hemangioendothelioma							1				
Popliteal L.N. - Hemangiosarcoma						1					

*p < 0.05.

This tumor, hemangiosarcoma, has a range of 0 to 1.4 percent according to CR data base for male CD-1 mice (circa 1985). More recent historical control data from the Assert Peer Review (SAP review) dated March 4, 1987 shows that hemangiomas/hemangiosarcomas occur spontaneously in male CD-1 mice at upper incidences varying between 3.3 and 13.3 percent. The observed incidences of hemangiosarcomas in male CD mice in the cyanazine study are within the range of historical control data of several laboratories. Additionally, the occurrence of hemangiosarcoma in males lacks a a clear dose-response relationship, which cannot be fully justified by a competing toxicity explanation. Since no splenic hemangiosarcomas were identified in males fed dietary concentrations of 250 and 1000 ppm and no tumors of this type, of this site, were recorded in any females fed the test compound, it is concluded that the statistically significant incidence at 10 ppm is a chance occurrence and is not compound-related. Table I summarizes the occurrence of hemangiosarcomas in the study. In male mice, the total percentages were 3, 12*, 4, 2, and 2 for the 0, 10, 25, 250, and 1000 ppm groups, respectively.

[redacted] Similarly, the statistically significant increase at 10 ppm is not considered compound-related.

Classification: Core-Minimum

*p < 0.05.

Table I

Total Number of Hemangiosarcomas in Various Organs in Cyanazine Mouse Study

	Males					Females				
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
No. Examined	100	50	50	50	50	100	50	50	49	50
Hemangiosarcoma										
Liver	1	2	0	0	0	0	0	1	0	0
Uterus	0	0	0	0	0	1	0	0	0	1
Lymph nodes	0	0	0	0	1	0	0	0	0	0
Subcutis	0	0	0	1	0	0	1	0	0	0
Thoracic wall	1	0	0	0	0	0	0	0	0	0
Spleen	1	4*	2	0	0	1	0	0	0	0
Dose (ppm)	0	10	25	250	1000	0	10	25	250	1000
Total Hemangiosarcomas	3	6	2	1	1	2	1	1	0	1
Number Examined	100	50	50	50	50	100	50	50	49	50
Total Percentage	3	12*	4	2	2	2	2	2	0	2

*$p < 0.05$.

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NON-ACUTE TOX PROFILE FOR: Cyanazine

TOX NO. - 188C SHAUGHNESSEY #: 100101 CAS REG #: 21725-46-2

STUDY	SPECIES	YR	GR	SYS NOEL	SYS LOEL	ONCO NOEL	ONCO LOEL	MAT NOEL	MAT LOEL	REPROD/ DEV NOEL	REPROD/ DEVLOEL	MUTA
Develpmental Tox. w post natal	rat	85	M					? mg/kg	5 mg/kg	5 mg/kg	25 mg/kg	
Develpmental Tox. w post natal	rat	85	M					? mg/kg	5 mg/kg	? mg/kg	5 mg/kg	
Developmental Toxicity Study	rabbit	82	M					1 mg/kg	2 mg/kg	1 mg/kg	2 mg/kg	
Developmental Toxicity Study	rabbit	86	M					? mg/kg	96 mg/kg	573 mg/kg	955 mg/kg	
Feeding/oncogenic-2 year	mice	81	M	?	10 ppm	> 1000 ppm						
Feeding-1 year	dog	86	M	25 ppm	100 ppm							
Mutagenic-chromosome aberr.	human l	87	A									-
Mutagen-gene mutation TK locus	L5178Y/	86	A									+
Mutagenic-unscheduled DNA synt	rat hep	?	A									+
Mutagenic-(HGPRT)	CHO cel	87	A									-

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U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/SACB
TOX ONELINERS

TOXCHEM NO. 188C- 2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile FILE LAST PRINTED: 02/08/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(a) eeding/oncogenic-2 year pecies: rat unstall Labs (England) 970	Bladex		Systemic NOEL = 12 ppm; Systemic LEL = 25 ppm (body wt. reduction). Oncogenic NOEL > 50 ppm (HDT). Doses tested = 12, 25, 50 ppm.		000811
83-1(a) and 83-2(b) eeding/oncogenic-2 year pecies: mice ittingbourne Res. Center BGR 81.171; 12/81	Bladex tech Batch 8-21-0-0	247295 247298	Carcinogenic NOEL > 1000 ppm (HDT). Systemic NOEL <= 10 ppm (LDT) (decreased body weight -- both sexes). Doses tested = 0, 10, 25, 1000 ppm CD strain.		Minimum 001884
83-1(a) and 83-2(a) eeding/oncogenic-2 year pecies: rat impson, BJ, & Dix LGR.0018.73; 7/73	Bladex (DW 3418) Batch No . FC 5097 97% pure	251954 251955 251956	Levels tested in Carworth Farm E. strain -- 0, 1, 3 and 25 ppm. Oncogenic NOEL -- inadequate data; Systemic NOEL -- inadequate data.		Supplementary 004221
83-1(a) and 83-2(a) eeding/oncogenic-2 year pecies: rat unstall Labs (England) LGR0063.70; 1970	Bladex (DW 3418) Batch No . FC 5097 97% pure	251949 251950 251951 251952 251953	Levels tested in Carworth Farm E strain -- 0, 6, 12, 25 and 50 ppm.		Invalid 004221
83-1(b) eeding-2 year pecies: dog unstall Labs (England) 970	Bladex		Systemic NOEL = 50 ppm; Systemic LEL = 100 ppm (reduced growth rates and liver weights in females).		000811
83-1(b) eeding-1 year pecies: dog azleton Lab America 160-104; 12/30/86	Cyanazine	40081901 40229001	Systemic NOEL = 25 ppm. Syst. LEL = 100 ppm based on reduced body wt. & body wt. gains, elevated platelet counts, reduced levels of total protein albumin & calcium in males and females. There was a slight, not stat. sig. decr. in spleen wts & incr. in liver wts in females, & incr. in liver wts & decr. in testes wts in males. No gross or microscopic finding related to treatment were noted. Levels tested: 0, 10, 25, 100 & 200 ppm mixed in the diet of beagles.		Minimum 006350

91

1970

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U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/SACB
TOX ONELINERS

TOXCHEM NO. 188C- 2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile FILE LAST PRINTED: 02/08/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(a) Developmental Toxicity Study Species: rat West Hollow Res. Center 61230; 12/81	Bladex tech	070584 071285	Teratogenic NOEL = 10 mg/kg/day; Teratogenic LEL = 25 mg/kg/day elevated incidence of anophthalmia & microphthalmia). Maternal NOEL = 2.5 mg/kg; Maternal LEL = 10 mg/kg (reduction in body weight). Fetotoxic NOEL => 25 mg/kg (HDT). Addendum to report. Doses tested = 0, 1.0, 2.5, 10.0, 25.0 mg/kg -- Fischer 344 strain.		Supplementary 001418 002446 003358
83-3(a) Developmental Toxicity Study Species: rat Research Triangle Inst. 311-2564; 5/16/83	Bladex tech (98.5%)	072838 071738	Teratogenic NOEL => 30 mg/kg/day (HDT). Maternal NOEL = 3.0 mg/kg/day; Maternal LEL = 30 mg/kg/day (HDT). Fetotoxic NOEL => 30 mg/kg/day (HDT). Levels tested = 0, 1, 3 and 30 mg/kg/day by gavage in SD-CD rats. Reclassified as Supplementary data due to disparity in findings with the Argus Res.' study (#619-002P).		Supplementary 003358 Minimum 004077 Supplementary 004491
83-3(a) Developmental Toxicity Study Species: rat Argus Research Labs 612-002P; 11/20/84	Bladex tech	256693 256694 256695 256696	Dose-range finding study: Maternal NOEL < 10 mg/kg/day (LDT) for both Sprague-Dawley and Fischer-344 rats. Fetotoxic and teratogenic NOEL not determined due to inadequate data available. Dose tested: 0, 10, 50, 100, 150 and 200 mg/kg/day.		Supplementary 004491
83-3(a) Develpmental Tox. w post natal Species: rat Argus Research Labs 619-002; 4/18/85	Bladex tech	257867	Dose levels: 0, 5, 25 & 75 mg/kg/day in Fischer-344 rats by gavage. C-section data: Developmental toxicity NOEL < 5 mg/kg/day (LDT); Teratogenic NOEL = 5 mg/kg/day. Post-natal data: Developmental toxicity NOEL = 5 mg/kg/day (LDT); Teratogenic NOEL = 5 mg/kg/day. Combined C-section and postnatal: Maternal NOEL < 5 mg/kg/day (decreased body cut and dose related increases in clinical manifestations). Developmental Toxic NOEL < 5 mg/kg/day (Significant alteration in skeletal variations). Teratogenic NOEL = 5 mg/kg/day; Teratogenic LEL = 25 mg/kg/day (abnormal development of the diaphragm; anophthalmia/ microphthalmia).		Minimum 004525

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TOXCHEM NO. 1880- 2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile FILE LAST PRINTED: 02/08/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(a) Develpmental Tox. w post natal Species: rat Argus Research Labs 6109-002; 4/85	Bladex tech	257867	Dose levels: 0, 5, 25 and 75 mg/kg/day in Fischer-344 rats by gavage. C-section data: Developmental toxicity NOEL < 5 mg/kg/day (LDT); Teratogenic NOEL = 5 mg/kg/day. Post-natal data: Developmental toxicity NOEL = 5 mg/kg/day (LDT); Teratogenic NOEL = 5 mg/kg/day. Combined C-section and Post-natal data: Maternal NOEL < 5 mg/kg/day (decreased body cut and dose related increases in clinical manifestatio). Developmental Toxic NOEL <= 5 mg/kg/day (significant alterations in skeletal variations). Teratogenic NOEL = 5 mg/kg/day; Teratogenic LEL = 25 mg/kg/day (abnormal development of the diaphragm); anophthalmia /microphthalmia). The 75 mg/kg/day exhibited cleft palate, exencephaly, and dilated brain ventricles.		Minimum 004525
83-3(b) Developmental Toxicity Study Species: rabbit IBT 8530-11112	Bladex tech	241970	IBT Invalid. Clement Associates. Contract No. 68-01-5824; 10/7/81.		000813 Invalid 001554
83-3(b) Developmental Toxicity Study Species: rabbit IBT J238; 2/78	Bladex tech		IBT Invalid		Invalid 000813
83-3(b) Developmental Toxicity Study Species: rabbit Sittingbourne Res. Center 721/81; 11/82	Bladex tech (98%)	071382	Teratogenic NOEL => 4 mg/kg/day)HDT). Maternal NOEL = 1 mg/kg (LDT); Maternal LEL = 2 mg/kg (anorexia, decreased body wt.). Fetotoxic NOEL = 1 mg/kg (LDT); Fetotoxic LEL = 2 mg/kg (increased # post implantation losses, decreased # live fetuses/dam, slight decrease in body weight). NOTE: At 4 mg/kg -- significant increase in # dead fetuses/dam and increased incidence of anomalies i.e. domed head in 4 fetuses (2 litters). Doses tested: 0, 1.0, 2.0, 4.0 mg/kg New Zealand white strain.		Minimum 002703
83-3(b) Developmental Toxicity Study Species: rabbit Wil Research Lab 03002; 2/86	Bladex 4L (43% ai by wt.)	261601	Maternal NOEL < 0.2 ml/kg (LDT). Developmental Toxicity NOEL > 0.2 ml/kg (LDT; increased skeletal variations). Dose levels: 0.2, 0.6, 1.2, and 2.0 ml/kg (approximately 105, 310, 620 and 1050 mg/kg) dermally to New Zealand rabbits from days 6-18 of gestation.		Supplementary 005053

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TOXCHEM NO. 188C- 2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile FILE LAST PRINTED: 02/08/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(b) Developmental Toxicity Study Species: rabbit Wil Research Lab 93002A; 2/86	Bladex 4L (43% ai by wt.)	261601	PILOT STUDY: Maternal NOEL and developmental NOEL not established (single dose used, inadequate number of dams, and no concurrent control group). Dose levels: 0.2 ml/kg (approx. 105 mg/kg) dermally to New Zealand rabbits from days 6-18 of gestation.		Supplementary 005053
83-3(b) Developmental Toxicity Study Species: rabbit Wil Research Lab 93003; WRC RIC-451; 6/20/86	Bladex 4L (43% ai)	263813	Maternal NOEL < 96 mg/kg (LDT; dermal irr.), decreased body wt. gain). Developmental Tox. NOEL = 573 mg/kg; Developmental Tox. LEL = 955 mg/kg (HDT; increased incidences of delayed ossification); A/D ratio: less than 1. Dose levels: 0, 0.2, 0.6, 1.2 and 2.0 ml/kg (0, 96, 286, 573, and 955 mg/kg) by dermal exposure in New Zealand rabbits.		Minimum 005331
83-4 Reproduction-3 generation Species: rat Hine Lab 1969	Bladex		NOEL > 80 ppm (HDT). Doses tested = 0, 3.9, 27, 80 ppm Long Evans strain		000811
83-4 Reproduction-2 generation Species: rat Wil Research Lab WIL 93001; 8/12/87	Bladex Tech. 100% a.i.	403600-01	Reproductive toxicity NOEL = 3.8 mg/kg/day Reprod. LOEL = 11.2 mg/kg/day (based on 75 ppm to mother). Parental (systemic) tox. LEL = 1.8 mg/kg/day (based on average intake) (LDT). Doses: 0, 25, 75, 150 & 250 ppm in Sprague-Dawley strain.		Supplementary 006597 Minimum 007804
82-1(a) Feeding-13 week Species: rat	Bladex		NOEL > 10000 ppm (HDT)		
82-1(a) Feeding-13 week Species: rat Tunstall Labs (England) 1968	Bladex DW4385 (metabolite XII)		Systemic NOEL = 200 ppm; SYstemic LEL = 800 ppm (HDT; non-degenerative, liver cell reaction). Doses tested = 0, 12.5, 50, 200, 800 ppm -- Carworth Farm E strain.		000811
82-2 Dermal-3 week Species: rabbit Stanford Research Inst. 168-19; 5/5/70	Bladex SD-15418	257868	Findings of hyperkeratosis and acanthosis occurred in both control and treated animals and may be related to the vehicle used.		Supplementary 004525

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	CORE/GRADE/ DOCUMENT#
82-4 Inhalation-3 month dust Species: rat IBT 8562-08627; 7/29/76	Bladex tech		IBT Invalid. Clement Associates. Contract No. 68-01-5824. 2/12/81. Accepted by EPA 2/24/81.		Minimum 000814 Invalid 001533
84-2(a) Mutagenic-Ames Species: salmonella Haskell Lab 458-87; 5/7/87	Bladex tech. (96%)	403047-03	Although as negative for inducing reversions in salmonella strains e exposed up to 5000 ug/plate, procedural and reporting deficiencies exist.		Unacceptable 007893
84-2(b) Mutagenic-bone marrow cells Species:	Bladex		Negative		000814
84-2(b) Mutagenic-chromosome aberr. Species: human lymphocytes Haskell Lab 528-87; 6/18/87	Bladex tech. 96%	40304705	Negative for inducing aberrations in human peripheral lymphocytes exposed in vitro to cytotoxic levels (250-350 ug/ml).		Acceptable 007893
84-4 Mutagenic-dominant lethal test Species: mice	Bladex		Negative		000814
84-4 Mutagenic- host med. Species:	Bladex		Negative		000814
84-4 Mutagen-gene mutation TK locus Species: L5178Y/TK Mamm cells West Hollow Res. Center 61282; 8/12/86	Bladex tech (% not stated)	00165051	Positive for induced mutation at the thymidine kinase locus in mouse lymphoma cells treated to the limit of solubility (1000, 1600 ug/ml).		Acceptable 007893
84-4 Mutagenic-unscheduled DNA synt Species: rat hepatocyte Haskell Lab HLR 347-87	Bladex tech. 96%	403047-02	Positive for unscheduled DNA synthesis in rat hepatocyte cultures at 50 uM and above.		Acceptable 007893

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-4 Mutagenic-(HGPRT) Species: CHO cells Haskell Lab 747-86; 1/7/87	Bladex tech 96%	40304704	Negative for inducing gene mutation in repeat assays treated up to cytotoxic limits (1.4 mM).		Acceptable 007893
85-2 Dermal absorption Species: rat Research Triangle Inst. RTI/3134/01F; 12/84	Bladex-C14 4L	256324	Study unacceptable due to excessive loss of material.		Unacceptable 004303
85-2 Dermal absorption Species: rat Research Triangle Inst. WRC RIR 427; 2/86	Bladex-C14	261602	Dermal absorption is minimal, reaching a max. of 2% of the applied dose.		Acceptable 005053
Peer Review Species: 4/19/87	Cyanazine		Developmental toxicity - Peer review response to SAP's issues related to toxic endpoints.		008049
81-1 Acute oral LD50 Species: rat	Bladex technical		LD50 = 334 mg/kg.	2	Minimum 000816
81-1 Acute oral LD50 Species: rat IBT 8530-9471; 11/12/76	Bladex 53.4 % Atrazine 25.3 % WP 2:1 comb.		LD50 = 384 (266-465) mg/kg (M & F). LD 40 = 480 (348-662) mg/kg (M). LD50 = 270 (201-363) mg/kg (F). Hypoactivity, salivation, rhinitis, lacrimation, diarrhea and hypothermia. Doses tested: 118.5, 177.8, 266.7, 400, 600, 900, 1350, and 2025 mg/kg, SD strain.	2	Guideline 000815
81-1 Acute oral LD50 Species: rat Wil Research Lab 1104-77; 12/23/77	Bladex 28.7 % Atrasine 13.6 %	240858	LD50 = 0.33 (0.25-0.43) ml/kg (M). LD 50 = 0.28 ml/kg (F). Red discharge from eyes, depression and labored respiration. Doses tested: 0, 0.1, 0.3, 1.0, 3.0, 5.0 and 10.0 ml/kg. SD strain.	2	minimum 000812
81-1 Acute oral LD50 Species: rat Stillmeadow Inc. 3552-84; 4/3/85	Bladex 43 %	257667	LD50 = 516 (445.2-603.7) Female. LD50 = 913 (472-1766.2) Male. Doses tested- 500, 1000, 2000, and 5050 mg/kg.	3	Guideline 006252

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TOXCHEM NO. 188C· 2-[[4-Chloro-6-(ethylamino)-s-triazin-2-yl]amino]-2-methylpropionitrile FILE LAST PRINTED: 02/08/91

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-1 Acute oral LD50 Species: rat Tillmeadow Inc. 192-83; 2/8/84	2,4-Chloro-6-(ethylamino)-s-triazin-2-yl-amino-2-methyl-prcpionitrile 17.18%; Monosodium acid methanearsonate 37.80%	253332	LD50 (M) = 507 mg/kg. LD50 (F) = 477 mg/kg. LD50 (comb.) = 494 mg/kg.	2	Guideline 006472
81-2 Acute Dermal LD50 Species: rabbit	Bladex technical		LD50 > 2000 mg/kg.	3	Minimum 000816
81-2 Acute Dermal LD50 Species: rabbit BT 530-9471; 11/12/76	Bladex 53.4 % Atrazine 25.3 % 2:1 comb.		LD50 > 2000 mg/kg (only dose tested). No deaths; mild edema, very slight erythema. New Zeland White strain.	3	Minimum 000815
81-2 Acute Dermal LD50 Species: rabbit Hil Research Lab 104-77; 12/19/77	Bladex 28.7 % Atrazine 13.6 %	240858	LD50 > 1960 Mg/kg (only dose tested). 1/ 3 died, slight to moderate erythemia and slight edema-24 hour exp. NZW strain.	3	Minimum 000812
81-2 Acute Dermal LD50 Species: rabbit Tillmeadow Inc. 553-84; 1/15/85	Bladex 43 %	257667	LD50 > 2020 mg/kg (only dose tested)	3	Guideline 006252
81-2 Acute Dermal LD50 Species: rabbit Tillmeadow Inc. 199-84; 2/8/84	Bladex 17.18 % Monsodium acid methanearsonate 37.8 %	253332	LD50 > 2010 mg/kg.	3	Guideline 006472
81-3 Acute inhalation LC50 Species: rat BT 562-08628; 5/4/76	Bladex technical		LC50 > 2.46 mg/L/1 hr. (Only dose tested). Ptosis, hyperactivity and salivation. Charles River strain.	3	Minimum 000814

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-3 Acute inhalation LC50 Species: rat	Bladex 80 WP		LC50 > 4.9 mg/L. No deaths.	3	Minimum 000816
81-3 Acute inhalation LC50 Species: Rat IBT 3562-9472; 11/12/76	Bladex 53.4 % Atrazine 25. 3 % 2:1 comb.		LC50 > 2.25 mg/L/4 hrs. Ptosis, salivation, lacrimation: no deaths Charles River strain.	3	Minimum 000815
81-3 Acute inhalation LC50 Species: rat West Hollow Res. Center 61230; 8/29/83	Bladex 97 %	252504	LC50 > 809 Mg/L	4	Guideline 006135
81-3 Acute inhalation LC50 Species: rat Stillmeadow Inc. 3557-84; 3/21/85	Bladex 43 %	257667	Gravimetrically measured concentration -2.94 mg/L. Analytic measure 2.82 mg/L. LC50 > 2.82 mg/L.	3	Guideline 006252
81-3 Acute inhalation LC50 Species: rat Stillmeadow Inc. 5195-83; 3/23/84	Bladex 17.18 % Mono.sodium methanerasonate 37.8 %	253332	LC50 > 3.0 mg/L.	3	Guideline 006472
81-3 Acute inhalation LC50 Species: rat West Hollow Res. Center 61230; 1/20/83	Bladex tech. a.i. % N/A	001376-61	LC50 (M&F) > 809 mg/m3 analytical conc.. The purity of the test article was not provided.	3	Supplementary 007953
81-4 Primary eye irritation Species: rabbit IBT 3530-9471; 11/12/76	Bladex 53.4 % Atrazine 25.3 % 2:1 comb.		Reversible corneal opacity before 7 days. Draize scores in unwashed eyes at 1, 24, 48 and 72 hours = 32.3, 27.4, 19.7 and 10/110. Dose tested: 100 mg- New Zeland White strain.	2	Minimum 000815

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-4 Primary eye irritation Species: rabbit Wil Research Lab 1104-77; 12/23/77	Bladex 28.7 % Atrazine 13.6 %	240858	Erythema and swelling. No opacity. All eyes normal by day 4. Dose tested: 0.1 ml-New Zeland White strain.	3	Minimum 000812
81-4 Primary eye irritation Species: rabbit Stillmeadow Inc. 3554-84; 1/23/85	Bladex 43 %	257667	Irritation cleared by 7 days.	3	Guideline 006252
81-4 Primary eye irritation Species: rabbit Stillmeadow Inc. 3193-83; 2/18/84	Bladex 17.18 % Mono sodium methanearsonate 37.8 %		24 hours: 2/6 iris irritation (SC.5). 6/6 and 2/3 redness(SC.1) 6/6 and 1/3 chemosis (SC.1+2)/ 2/6 discharge (SC.1) 72 hours clear except 1/3.		Guideline 006472
81-5 Primary dermal irritation Species: rabbit IBT 8530-947i; 11/12/76	Bladex 53.4 % Atrazine 25.3 % 2:1 comb.		PIS = 1.0/8.0 at 24 hours. Erythema cleared by 72 hours. Dose tested: 500 mg- New Zeland White strain.	4	Minimum 000815
81-5 Primary dermal irritation Species: rabbit Wil Research Lab 1104-77; 12/23/77	Bladex 28.7 % Atrazine 13.6 %	240858	PIS = 0.42/8.0. Very slight erythema and edema. Clear in all but 1/6 intact sites by 72 hours. Dose tested: 0.5ml-NZW strain.	4	Minimum 000812
81-5 Primary dermal irritation Species: rabbit Stillmeadow Inc. 3555-84; 1/22/85	Bladex 43 %	257667	Maximum irritation score = 0.3.	4	Guideline 006552
81-6 Dermal sensitization Species: guinea pig IBT 8530-947i; 11/12/76	Bladex 53.4 % Atrazine 25.3 % 2:1 comb.		Negative (no irritation after induction and challenge doses). Dose tested: 9 induction doses + 2 challenge doses. Material tested as 10 % aqueous suspension.		Minimum 000815

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
81-6 Dermal sensitization Species: guinea pig Wil Research Lab 1104-77; 3/23/78	Bladex 28.7 % Atrazine 13.6 %	240858	Erythema and edema observed after the first sensitizing dose. Dosing: 1 ml once/week fo three weeks-challenge dose after 2 week rest period. Hartley albino strain, males and females.		Supplementary 000812
81-6 Dermal sensitization Species: guinea pig Stillmeadow Inc. 3556-84; 2/26/85	Bladex 43 %	257667	Non-sensitizer.		Guideline 006252

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