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

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MAR -6 1991

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Cyanazine - EPA Registration No. 352-475 - 6(a)(2) Data - 2-Year Chronic Toxicity/Oncogenicity Rat Study with Cyanazine Technical - Reregistration and Special Review Branchs' Referral

Project No.: 0-80066
Caswell No.: 188C
MRID No.: 41509902
Record No.: 268381

FROM: William Dykstra, Ph.D. William Dykstra 2/13/91
Review Section I
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

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

and

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

THRU: Roger Gardner, Acting Section Head
Review Section I Roger Gardner 2/14/91 KB 2/24/91
Toxicology Branch I - Insecticide, Rodenticide Support
Health Effects Division (H7509C)

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Requested Action

Review 2-year rat feeding study submitted as 6(a)(2) data by the registrant.

Conclusion and Recommendation

1. Cyanazine may be a mammary gland carcinogen in female Sprague-Dawley rats producing statistically significant increases in adenocarcinomas at dosages of 5, 25, and 50 ppm (HDT). Additionally, there was a statistically significant trend for adenocarcinomas with dose in the study. The pairwise comparison p-values, based on "crude proportions," were 0.0535, 0.0021, and 0.013 for the 5, 25, and 50 ppm dose levels, respectively.

   These p values, when adjusted for survival disparity by the statistics teams, may be of greater statistical significance.

   Additionally, in the 5, 25, and 50 ppm groups there were one and two additional tumor-bearing animals with fibrosarcoma (5 and 50 ppm) and one animal with carcinosarcoma at 25 ppm. These tumor-bearing animals, together with those bearing adenocarcinomas, represent the total number of tumor-bearing animals with malignant tumors. Therefore, the total number of malignant tumor-bearing animals was 5, 7, 13, 20, and 16 for the 0, 1, 5, 25, and 50 ppm groups, respectively.

   The percent incidences of tumor-bearing animals with adenocarcinoma was 8, 11, 20, 31, and 23 percent for the 0, 1, 5, 25, and 50 ppm groups, respectively. The slight decrease in adenocarcinomas in the 50 ppm group in comparison with the 25 ppm group can be attributed to competing toxicity (e.g., weight-gain decreases at 50 ppm). In any event, the trend was highly significant.

2. In order to further evaluate the results, Toxicology Branch (TB) considered Charles River (CR) Laboratories historical control data from 11 studies from 1977 to 1985 (the best data available). The range of adenocarcinoma in the Sprague-Dawley rat in 11 studies was from 0 to 16.0 percent with a mean of 7.4 percent.

It can be seen that in the cyanazine study, the concurrent control incidence of 8 percent is almost identical to the CR mean and also falls within the historical control range.

Additionally, the incidences of 20, 31, and 23 percent for the 5, 25, and 50 ppm groups exceed the CR historical control range of 0 to 16 percent for adenocarcinomas.

In summary, based on the available data, cyanazine may be considered a mammary gland carcinogen at 5, 25, and 50 ppm in this study.

These findings possibly exceed Special Review criteria. Additionally, TB recommends against reregistration of cyanazine, pending a formal Peer Review and possible quantification of cancer risk.

3. The registrant must resubmit the 10 historical control studies which were presented as incidences occurring between 1 year and termination. Each study needs to be reevaluated, and the number of animals examined must exclude those dying before the appearance of the first malignant tumor or adenocarcinoma. This is standard practice. This is needed so that the denominator (number of animals examined) will not be biased by selection after 1 year but will include all animals at risk. This new statistical evaluation may or may not alter the current percentages of malignant tumors observed in the 10 studies.

   At this point, the comparison of the historical control data incidences provided by the registrant is unusable either in comparison with the "crude proportions" (in which all 62/sex/group were used) or with the statistical package soon to be completed by the SACB Biostatistics Team.

4. With respect to non-neoplastic lesions, historical control data are needed for:

   a. Granulocytic hyperplasia of bone marrow in males.

   b. Extramedullary hematopoiesis of spleen in males.

   c. Demyelination of the sciatic nerve in females.

   Please include incidences and grades of lesion.

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

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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)*	X	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		X	
	Electrolytes:		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	Rectum*	XX	Testes*	X	Mammary gland*
XX	Liver*	X	Epididymides	X	Parathyroids*
	Gall bladder*	X	Prostate	X	Thyroids*
X	Pancreas*	X	Seminal vesicle		Other
	Respiratory	X	Ovaries	X	Bone*
X	Trachea*	X	Uterus*	X	Skeletal muscle*
X	Lung*	X	Vagina	X	Skin
X	Nose			X	All gross lesions and masses

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.)

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*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

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)[o]	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)

o = 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.

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."

	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

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	

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

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 29 through 35 are not included.

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Reviewed By: William Dykstra, Ph.D. William Dykstra 11/29/90
Section I, Toxicology Branch I - IRS (H7509C)
Secondary Reviewer: Roger Gardner, Section Head Roger Gardner
Section I, Toxicology Branch I - IRS (H7509C) 2/11/91

008282

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)*		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
X	Stomach*	X	Lymph nodes*		(3 levels)
X	Duodenum*	X	Spleen*	X	Pituitary*
X	Jejunum*	X	Thymus*	X	Eyes (optic
X	Ileum*		Urogenital		nerve)
X	Cecum*	XX	Kidneys*		Glandular
X	Colon*	X	Urinary bladder*	X	Adrenals*

X		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Pectum*	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						NCR	NCR	NCR	NCR	NCR
Heart				D	D					
Liver	NCR	NCR	NCR	NCR	NCR					
Testes										
Kidneys			D	D	D					D
Adjusted (To Terminal Body Weight)										
Brain				I	I				I	I
Heart					I					I
Liver										I
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]).

Females had an increased incidence of ulcerated skin observed at necropsy in the 250 and 1000 ppm groups in 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.

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)	NOEL = 250 ppm LEL = 1000 ppm									
	12 12%	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)	NOEL = 25 ppm LEL = 250 ppm									
	10 10%	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)	0	10	25	250	1000	0	10	25	250	1000
Number of Animals Examined	100	50	50	50	50	100	50	50	49	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	10	25	250	1000	Females 0	10	25	250	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.

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

Classification: Core-Minimum

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*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

*$p < 0.05$.

57395:I/C:DRAFT:LHED-10:KENCO:11/21/90:12/21/90:DD:JH:DD
R:57395:Dykstra:LHED-10:KENCO:11/27/90:12/27/90:CL:vo:ek:de

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