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

004221

MEMORANDUM:

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: EPA Reg. No. 201-298; Cyanazine (Bladex)®: Oncogenicity in Rats. Caswell No. 188C.

201-351

TO: Robert Taylor
Product Manager (25)
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D. C. F. Chaisson 1/4/85
Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: George Z. Ghali, Ph.D. G. Ghali 12/31/84
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant: Shell Oil Company
1025 Connecticut Avenue
Washington, D.C. 20036

Action Requested:

Review and evaluation of two oncogenicity studies in the rat.

Conclusions and Recommendations:

Two chronic feeding/oncogenicity studies were submitted by the registrant. The two studies were evaluated by Dynamac Corporation, and further evaluated by the Toxicology Branch of the Hazard Evaluation Division. The following conclusions were made:

1. Simpson, B.J. and Dix, K.M. Toxicity studies on the s-triazine herbicide Bladex: Second 2-year oral experiment in rats. Unpublished Report No. TLGR.0018.73. Prepared by Shell Research Limited, London. Dated July 1973. Accession numbers 251954, 251955, 251956.

This study is considered unacceptable for the assessment of the potential chronic toxicity and oncogenicity of Bladex primarily due to numerous deficiencies resulting in a decreased power of the test to detct possible compound-related effects. These deficiencies involved the following: limited number of animals used per test group; the lack of overt toxicity in

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the dosed animals as compared to controls indicated that the animals could have tolerated higher dietary levels of the test material; convulsions observed in the control and dosed animals suggested a possible cross dosing of animals or errors in the formulation of diets; dietary doses administered were not confirmed by chemical analyses; limited numbers of tissues per animal were subjected to histologic examination; results of gross examinations and histologic findings for non-neoplastic lesions were not adequately reported; urinalyses were not performed; and clinical blood chemistry analyses were limited. the study is classified as Core-supplementary data.

2. Walker, A.I.T. and Thorpe E. 1970. Toxicity studies on the s-triazene herbicide Bladex (DW 3418): 2-year oral experiment with rats. An unpublished report prepared by Tunstall Laboratory, submitted by Shell Research Limited, London. (TLGR 0063.70).

Malley, L.A., VanGelder, G.A., Chai, E.Y., Patterson, D.R. 1983. Statistical analysis of tumor data from two Bladex® chronic studies in rats (TLGR 0063.70 and TLGR 0018.73) An unpublished report prepared by Shell Westhollow Research Center, Houston Texas.

Linnett, S. Historical data - Tinstall CFE rats, classification of adrenal modullary tumors. An unpublished report prepared by Turnstall Laboratory and submitted by Shell Oil Company. Dated July 12, 1984.

Accession numbers 251949, 251950, 251951, 251952, 251953.

This study is considered unacceptable for the assessment of the potential chronic toxicity and oncogenicity of Bladex in rats primarily due to numerous deficiencies resulting in a decreased capability of the test to detect possible compound-related effects. These deficiencies involved the following: the lack of overt toxicity in the dosed animals indicated that the animals could have tolerated higher dietary levels of the test material; limited number of tissues per animal were subjected to histologic examination; tumor incidences presented were not supported by the data and were calculated based on the number of animals tested rather than the number of specific tissues histologically examined; limited number of animals were available in the mid-dose groups for adequate evaluation; results of gross examination and histologic findings for non-neoplastic lesions were not adequately reported; clinical observations were not reported; and limited hematology, clincal blood chemistry, and urianlyses data were presented. The study is considered invalid and irrepairable.

TWO-YEAR FEEDING STUDY IN RATS

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ACCESSION NUMBER: 251954-955-956

Simpson, B.J. and Dix, K.M. Toxicity studies on the s-triazine herbicide BLADEX: Second 2-year oral experiment in rats. Unpublished Report No. TLGR.0018.73. Prepared by Shell Research Limited, London. Dated July 1973.

TEST CHEMICAL:

The test material was identified as BLADEX (DW 3418); 2-(4-chloro-6-ethylamino-s-triazin-2-ylamino)-2-methylpropionitrile from batch No. FC 5097 with a purity of >97 percent. The sample was supplied by Degussa, Frankfurt, West Germany, and was also used in the first two-year oral toxicity experiment.

PROTOCOL:

1. Male and female Corworth Farm E. strain, bred in house were used in the study. The animals were about 5 weeks old (born within 5 days of each other) and were individually caged and maintained under specific-pathogen free conditions. The environmental conditions of the animal room were not specified. At the beginning of the study the mean body weights of males and females were 90-103 g and 93-94 g, respectively.

2. The animals were randomly assigned to 4 groups; the control group consisted of 48 animals/sex, and each of the dosed groups consisted of 24 animals/sex. In addition, 10 animals/sex/group were included 4 weeks later and 5 animals/sex/group sacrificed after 6 and 12 months.

3. The dosed groups received diets containing 1, 3, and 25 ppm of test material. The control groups received the untreated diet, Powdered Diet 86 from Scientific Products Farm, Ash, Kent, England. The test diet preparation method was not described and the frequency of diet preparation throughout the study was not reported.

4. The "health and behavior of all animals were observed daily." Animal body weights and food consumption were determined weekly for the first 13 weeks and at 4-week intervals thereafter.

5. Blood samples for hematologic and clinical chemistry determinations were collected from 5 animals/sex/group at 6 month and 1 year interim sacrifice, and from all surviving animals at final sacrifice. Hematologic examinations included hemoglobin, hematocrit, erythrocyte, leucocyte and differential leucocyte counts. Clinical chemistry

determinations included serum urea, total protein, and sodium, potassium, and chloride ions, and plasma alkaline phosphatase and glutamic pyruvic transaminase activities; serum proteins were determined after electrophoretic separation.

6. Gross examination was conducted on all animals found dead, sacrificed in extremis or sacrificed at 6, 12, and 24 months. The following fresh organ weights from sacrificed animals were determined: brain, heart, liver, kidneys, and testes.

7. Histologic examination of "a wide range of tissues" was conducted, but the tissues examined were not specified.

8. Body and organ weights data were analyzed statistically using the initial body weight as a covariate in covariable analysis wherever a significant relationship existed. Hematology and clinical chemistry date were examined using analysis of variance. The Student "t" test was used to determine significant differences between dosed and control groups.

   Tumor incidence data were adjusted for animal mortality by the life table analysis of Cox, and a Bonferroni correction for multiplicity of tests was used.

RESULTS:

Diet Analyses: The concentration and stability of the test material in the test diets were not determined. Nonetheless, the authors reported that data from a more recent study indicated that the test material was stable at room temperature over a three year period.

Clinical Observations and Mortality: Data for daily clinical observations were not presented in the report. The authors stated that "the general health and behavior of control and treated animals were similar throughout the study." In addition, approximately 3 months after the beginning of treatment "convulsions were observed in 42 percent of the animals and the incidence was not dose-related." An examination of individual pathology forms by this reviewer indicated the presence of limited information on the clinical history of each animal. Convulsions in individual animals were recorded for various intervals throughout the study, and no specific pattern could be determined. The compiled data for control and high dose groups indicated that the incidence of convulsions was much higher in males than females and were observed more frequently in males that died during the study as compared to animals surviving to the end of the study (Table 1). The cause of these convulsions in control and dosed male rats was not reported.

TABLE 1. Incidence of Convulsions in Rats Fed Bladex Containing Diets for Two Years

Test Group	Group Control	25 ppm
One Year Sacrifice		
Males	1/5	0/5
Females	1/5	0/5
Two Year Study		
Males	21/48	11/24
Females	1/48	0/24

There were no differences in survival among treated and control animals at 18 and 24 months (Table 2). Mortality for males and females at 18 months ranged form 13-33 and 15-25 percent, respectively. At the end of the study, mortality ranged from 50-75 percent for all groups (Table 2).

TABLE 2. Mortality in Rats Fed Diets Containing Bladex for Two Years

Test group/dose	Mortality at Months (percent) 18[a]	24
Males		
Control	12 (25)	36/48 (75)
1 ppm	6 (25)	15/24 (63)
3 ppm	3 (13)	14/24 (58)
25 ppm	8 (33)	15/24 (63)
Females		
Control	7 (15)	29/48 (60)
1 ppm	4 (17)	12/23 (47)
3 ppm	6 (25)	15/24 (63)
25 ppm	6 (25)	14/24 (58)

[a]Compiled by this reviewer.

Body Weights and Food Consumption: A significant decrease in mean body weights was noted in all dosed groups during the first 4-8 weeks of the study as compared to the controls (Table 3). Dosed animals recovered by week 13 except for the 25 ppm males which exhibited decreased body weights throughout month 12 of the study.

There were a few sporadic increases or decreases in food consumption between control and dosed groups throughout the study, but no time-or dose-related changes were noted.

Body weight and food consumption data for the animals sacrificed at 6 and 12 months indicated similar findings, although decreased mean body weight in high dose males was not observed for the 6 month sacrifice group.

TABLE 3. Mean Body Weights of Rats Fed Diets Containing Bladex for Two Years

Test Group/Dose	Mean Body Weight (g)				
Week	0	12	52	80	104
Males					
Control	99	395	494	475	391
1 ppm	103	390	497	463	366
3 ppm	99	385	489	464	373
25 ppm	90	377*	480*	469	383
Females					
Control	94	272	341	343	305
1 ppm	94	264	339	354	310
3 ppm	93	271	343	358	321
25 ppm	94	263*	339	336	300

* $p < 0.05$.

Hematology: The mid-dose male group had significantly lower hemoglobin content, erythrocyte count, and packed cell volume as compared to the control group. There were no other significant differences noted in hematology among the control and dosed groups at the end of the study. Similarly, there were no significant differences noted in hematology among the control and dosed groups at the one year sacrifice. Some significant differences were noted at the 26-week sacrifice and these parameters included: increased leucocyte counts in the low- and mid-dose females, and increased clotting time in all male and female dosed groups.

Blood Chemistry: At final sacrifice there was a significant increase in serum protein and a decrease in serum urea in the low-dose females, as compared to controls. There were no other differences noted among the

control and dosed groups. A significant decrease in serum urea content was also noted in the low- and high-dose female groups at the one year interim sacrifice. Serum glutamic pyruvic transaminase activities in the low- and mid-dose female groups were also lower than the control value at one year. There were no significant differences noted in blood chemistry parameters at the 26 week sacrifice except for an increase in serum protein of the mid-dose male group.

Gross Observations: Necropsy findings for the 6, 12, and 24 month sacrifice animals and animals dying during the study were not summarized and presented in the final report. Necropsy was not performed for the low- and mid-dose groups at the 12 month interim sacrifice. Although individual pathology sheets were present for all animals, except for those sacrificed at 6 months, and it appeared that gross findings were recorded for each animal at necropsy, it was not possible to validate and summarize the data because numerous pathology sheets were illegible. Consequently, an evaluation of gross pathology cannot be made for this study.

Organ Weights: At final sacrifice the mean (left) testes weights of dosed animals presented in the report were inconsistant with the individual animal data. The mean values for the testes weight and testes:body weight ratio calculated by this reviewer for the control, 1, 3, and 25 ppm groups were 3.38, 2.87, 2.55, and 2.80 g, and 0.91, 0.82, 0.74, and 0.76 g/100 g, respectively.

The mean weights of the following organs from males were significantly lower than control values at final sacrifice: mean brain weight of high-dose group, mean liver weights of the low- and mid-dose groups, and the mean kidney weight of the mid-dose group. However, there were no differences among dosed and control organ:body weight ratios. There were no other significant differences in absolute organ weights and organ:body weight ratios among control and dosed females.

At the 6-month sacrifice there were no differences between the mean organ weights of dosed and control animals. Significantly lower brain- and heart-to-body weight ratios were noted for the high dose males. This decrease is probably associated with the higher mean body weight of this group.

At the one year sacrifice there were no differences noted between the mean organ weights of dosed and control animals, except for significantly lower kidney weights in the high dose males and brain weights in high dose females. Significantly higher heart-to-body weight ratios were also noted for low- and high-dose males.

Histopathology: Only neoplastic lesions were summarized and presented in the report. Individual pathology sheets indicated that only a limited number of tissues from individual animals were examined. These included the major organs and occasionally the brain, pituitary, stomach, skeletal muscle, salivary glands, lymph nodes, urinary bladder, prostate, uterus, eye, thymus peripheral nerves, bone, etc.

It was not possible to summarize the data for non-neoplastic lesions because numerous pathology sheets were illegible. Moreover, summaries of histopathologic findings for individual animals could not be used because the total number of individual tissues examined for each group could not be determined. Frequently observed non-neoplastic lesions included the presence of variable amounts of calloid in the thyroid follicles, hemosiderin in the spleen, chronic nephrosis, and foci of hyperplasia of the adrenal cortex or medulla.

Data on neoplastic lesions are presented in Table 4. There were no significant differences noted in the incidence of neoplasms in males, although the mid-dose group had increased incidences of thyroid adenomas and adrenal pheochromocytomas. In females, the combined incidence of pituitary adenomas and carcinomas in the high-dose group was higher than the control group but was not statistically significant. Adrenal pheochromocytomas were also noted in the mid- and high-dose females and none were detected in the control animals.

TABLE 4. Summary of Neoplastic Lesions[a]

	Males				Females			
Neoplastic Lesion	0	1	3	25	0	1	3	25
Thyroid								
Adenoma	2/45[b]	0/16	4/20	2/25	9/48	5/18	1/20	3/27
Adrenal								
Adenoma	1/42	2/17	0/20	0/25	0/46	0/21	0/21	0/27
Pheochromocytoma	8/45	6/17	8/20	4/25	0/46	0/21	3/21	1/27
Pancreas								
Islet cell adenoma	3/45	0/17	2/20	0/25	4/46	0/21	0/20	0/25
Pituitary								
Adenoma	3/45	0/17	1/20	1/25	4/26	7/20	2/17	6/23
Carcinoma	1/45	0/17	0/20	0/25	1/26	0/20	0/17	2/23
Kidney								
Adenoma	1/45	1/17	1/20	0/25	0/48	0/21	0/20	0/27
Carcinoma	2/45	0/17	0/20	1/25	1/48	0/21	0/20	0/27
Mammary gland								
Fibroadenoma					16/49	9/22	8/20	9/25
Carcinoma					0/49	0/22	1/20	1/25

[a] Compiled by this reviewer from individual animal pathology sheets, and summary tables.

[b] Number of animals with tumor/total number of tissues examined.

DISCUSSION:

There were several major deficiencies noted in this study which limit its usefulness in determining the chronic toxicity and oncogenic potential of Bladex in rats. These deficiencies included:

1. A small number of animals/group were used, with only 24 animals in dosed groups and 48 in control groups instead of the 50 animals required by the EPA guidelines, which limited the power of the best to detect possible compound-related effects. This may have been the case with respect to adrenal pheochromocytoma in both sexes of rats and for thyroid adenoma in males and pituitary tumors in females.

2. The basis for dose selection was not documented or explained, although it was evident that the animals could tolerate higher doses than the ones used in this study, because no compound related effects were noted in any of the parameters tested. Moreover, an earlier chronic toxicity study with rats, which began only 14 months prior to this study, used higher doses of up to 50 ppm, and a subsequent chronic toxicity study with mice used a high dose of 1000 ppm. Consequently, it was not shown that a maximum tolerated dose (MTD) was used.

3. No explanation was presented for the increased incidences of convulsions in both control and dosed males. These signs may indicate poor animal husbandry or some other intrinsic factor such as cross dosing or errors in diet formulation. Diet analyses might have clarified this, however, these analyses were not conducted.

4. Results of gross examinations and histopathologic findings for non-neoplastic lesions were not summarized and presented. Although individual animal pathology sheets were presented, it was not possible to validate and summarize the data because numerous sheets, or parts thereof, were illegible. Moreover, these pathology sheets indicated that limited number of tissues were examined histologically with only the major organs and tissues systematically examined for each animal.

5. Urinalysis was not performed and several blood chemistry parameters were not investigated (e.g., calcium, phosphorus, glucose, creatinine, cholesterol, bilirubin, and creatine phosphokinase and serum glutamicoxaloacetic transaminase activities). Moreover, clinical chemistry was performed on only 5 animals at the 6 and 12 month sacrifice.

CONCLUSIONS:

This study is considered unacceptable for the assessment of the potential chronic toxicity and oncogenicity of Bladex primarily due to numerous deficiencies resulting in a decreased power of the test to detect possible compound-related effects. These deficiencies involved the following: limited number of animals used per test group; the lack of overt toxicity in the dosed animals as compared to controls indicated that the animals could have tolerated higher dietary levels of the test material; convulsions observed in the control and dosed animals suggested a possible cross dosing of animals or errors in the formulation of diets; dietary doses administered were not confirmed by chemical analyses; limited numbers of tissues per animal were subjected to histologic examination; results of gross examinations and histologic findings for non-neoplastic lesions were not adequately reported; urinalyses were not performed; and clinical blood chemistry analyses were limited.

CORE CLASSIFICATION:

Supplementary data.

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ONCOGENICITY/CHRONIC ORAL TOXICITY, RAT

ACCESSION NUMBER: 251,949 - 251,953.

Walker, A.I.T. and Thorpe E. 1970. Toxicity studies on the S-triazene herbicide Bladex (DW 3418): 2-year oral experiment with rats. An unpublished report prepared by Tunstall Laboratory, submitted by Shell Research Limited, London. (TLGR 0063.70).

Malley, L.A., VanGelder, G.A., Chai, E.Y., Patterson, D.R. 1983. Statistical analysis of tumor data from two Bladex® chronic studies in rats (TLGR 0063.70 and TLGR 0018.73). An unpublished report prepared by Shell Westhollow Research Center, Houston Texas.

Linnett, S. Historical data - Tinstall CFE rats, classification of adrenal modullary tumors. An unpublished report prepared by Turnstall Laboratory and submitted by Shell Oil Company. Dated July 12, 1984.

TEST MATERIAL:

The test material was identified as Bladex (DW 3418); 2-(4-chloro-6-ethylamino-6-ethylamino-s-triazin-2-ylamino)-2-methyl propiononitrile, batch No. FC 5097, and was supplied by Degussa, Frankfurt W. Germany. Purity was greater than 97 percent.

PROCEDURES:

1. Bladex was fed for two years to groups of 25 male and 25 female Carworth Farm E strain rats at dietary levels of 6, 12, 25, and 50 ppm. Control groups of 45 males and 45 females were fed untreated "Diet 86" powder. Water was available ad libitum. Additional groups of 18 males and 18 female controls and 9 males and 9 females at each of the above dose levels were sacrificed at 44 weeks. Additional groups of 6 control males and 6 control females and 3 animals of each sex and dose group were included in the study for sacrifice at 78 weeks. The study was terminated and all live animals sacrificed at the end of 2 years.

2. Although it was stated that the health and behavior of all animals were observed daily, only mortality data were available and there was no clinical observation data reported.

3. Body weights and food consumption were measured for each animal weekly for the first 13 weeks and at 4-week intervals thereafter.

4. Hematology and clinical chemistry studies were performed on all animals at the 44-week sacrifice, 78-week sacrifice and on animals sacrificed at study termination. Hematology parameters measured were hemoglobin, hematocrit, erythrocyte count and total and differential leukocyte counts. Clinical chemistry determinations included total serum protein, urea, alkaline phosphatase, serum glutamic-pyruvic transaminase, sodium, potassium and chloride.

5. Gross examinations were conducted on all animals that died, were sacrificed in extremis, or sacrificed on schedule (44 weeks, 18 months and 2 years).

6. Organ weights were determined at autopsy of animals at scheduled sacrifice. Weights of brain, heart, liver, spleen, kidneys, and testes were recorded.

7. Histopathologic examinations were conducted. The tissues to be examined were not stated in the protocol or procedures but it was evident from the data recorded that there was an incomplete examination of tissues.

8. Body and organ weight data were statistically analyzed by using the initial body weight as a covariate whenever a significant relationship existed. Hematology and clinical chemistry data were examined by analyses of variance; significance of differences between control and treatment groups were measured with the student 't' test.

9. Statistical analysis of tumor data was presented in a separate report. Data were adjusted for animal mortality by the life table analysis based on Cox (1972). A series of 2 x 2 tables for relevant time points were combined to obtain a single p-value. A Bonferroni correction for multiplicity of tests was applied by dividing the 5 percent significance value by the number of treatment group (0.05 ÷ 4). A trend test based on Tarone (1975) was also performed.

RESULTS:

Dietary Analysis: Dietary analyses were present for months 1-7, 9-14, 16, 17, 19, and 24. All values were within approximately 10 percent of nominal concentrations. Mean values for the doses were 5.7, 11.5, 24.7, and 50.5 ppm.

Observations and Mortality: Clinical observations were not presented in the report. The survival of animals at 18 and 24 months is summarized in Table 1. At 18 months, survival was between 76-90 percent in male groups and 74-90 percent in female groups. There was no compound related effect.

TABLE 1. Percent Survival of Rats Fed Bladex®[a]

Dose Group (ppm)	males/months 18	24	males/months 18	24
0	83	45	74	52
6	90	43	86	52
12	81	62	90	38[b]
25	81	67	81	67
50	76	67	81	61

[a] This data is for animals in the main groups of the study and does not take into account the deaths in the groups of animals scheduled for interim sacrifice. Compiled by this reviewer.

[b] This value is the actual data recorded but is not the value presented in the final report.

Body Weights and Food Consumption: Mean body weights were statistically lower in 50 ppm males compared to controls during the first 52 weeks of the study and in 25 and 50 ppm females compared to controls during the first 76 weeks of the study. However, at the end of the study there were no differences between test and control groups and the weight variations during the earlier part of the study were not considered by the authors to be of toxicologic importance. Among male and female groups there were only slight differences in weight gain or loss after week 24 of the study (Table 2). There were only sporadic changes (reductions) in food consumption in dosed animals throughout the study.

TABLE 2. Body Weights and Weight Change at Selected Intervals in Rats Fed Bladex®

Group	Body Weight (g) Weeks 0	24	52	76	104	Weight Change (g) Interval in Weeks 0-24	24-52	52-76	76-104
Males									
0	106	447	529	505	418	+341	+82	-24	-87
50	108	430*	506*	495	404	+322	+76	-11	-91
Females									
0	105	304	352	353	328	+199	+48	+ 1	-25
25	103	291*	333*	339*	313	+103	+42	+ 6	-26
50	108	288*	325*	325*	310	+108	+37	+10	-25

* Significantly different than control at $p < 0.01$.

Hematology: There were no treatment related effects on hematology parameters throughout the study. Hemoglobin was significantly higher in 25 ppm males and lower in 25 ppm females at terminal sacrifice than in respective controls, but values were within the normal range and not considered of toxicologic importance. There were no changes in any parameters at 11 or 18 months.

Clinical Chemistry: There were no effects on clinical chemistry parameters in dosed groups compared to controls.

Gross Pathology: The gross findings were not summarized and presented in the final report. Although individual necropsy sheets were present for all except two animals and it appeared that gross findings were recorded for each animal at gross examination, it was not possible to validate and summarize the data for evaluation because numerous pathology sheets or parts thereof were illegible. Consequently, an evaluation of gross pathology cannot be made for this study.

Organ Weights: There were no statistically significant changes in mean organ weights (brain, heart, liver, spleen, kidneys, and testes) of dosed animals compared to controls at the 44-week or 104-week sacrifice. Insufficient animals were available at the 18-month sacrifice to assess any effect on organ weights.

Histopathology: Pathology information sheets were present in the raw data for almost all animals entered in the study. Histopathology was not performed on the 9 males and 9 females in each of the 6, 12, and 25 ppm group that were sacrificed at 44 weeks; a few animals in various groups were missing or autolyzed and were not examined. Table 3 summarizes disposition of the animals. Histologic findings for individual animals were presented in an appendix; however, since only adverse findings were reported it could not be determined if a tissue was examined histologically and found to be normal or was not examined. The summary table of tumors listed the number of animals examined histologically and not the number of each target tissue examined histologically.

Subsequent to preparation of the final report in 1970 there were corrections of tumor data. Corrections were submitted on October 13, 1976 with amended tables and explanation of the corrections. An audit and validation/dated March 25, 1980 made further corrections. A summary of the tumor data for statistical analysis was submitted in the report dated December 5, 1983. Table 4 summarizes the tumor data as presented in this latter report.

According to the report, male rats fed 50 ppm Bladex had a significant increase in thyroid adenomas ($p = 0.0178$ using the Cox test); however, when the Bonferroni correction for multiple comparisons is applied to the data, a p-value of 0.0125 is required for statistical significance. When adenomas and carcinomas of the thyroid were combined there was no significant increase in any group compared to controls (Cox test). There was no significance in thyroid adenomas in males using the Tarone trend

TABLE 3. Animals Not Examined Histologically

Disposition	Dose Level (ppm) 0	6	12	25	50
Males					
autolyzed	2	2	0	1	1
missing	2	0	1	0	2
44 week - not examined	-	9	9	9	-
78 week - not examined	-	3	-	-	-
Total - not examined	4	14	10	10	3
No. started	66	33	33	33	33
No. with histopathology*	62	19	23	23	30
Females					
autolyzed	1	0	1	1	1
missing	2	1	0	0	0
44 week-not examined	-	9	9	9	-
78 week-not examined	-	-	-	-	-
Total - not examined	3	12	10	10	10
No. started	66	33	33	33	33
No. with histopathology*	63	21	23	23	32

* The actual number of specific tissues examined are indicated in Table 4.

test. When incidence of adrenal pheochromocytomas was compared between control males and 6 ppm males the p-value was 0.0488 using the Cox test; a p-value of 0.0525 was determined comparing mammary tumors in 0 and 25 ppm females. Based on these analyses the authors concluded that the tumors were not compound-related and that Bladex® was not an oncogen.

There was no summary of non-neoplastic lesions in the report and it was not possible to summarize and evaluate the data available because numerous pathology sheets or parts thereof containing this information were illegible. It was noted that "chronic nephrosis was almost universal in these rats".

TABLE 4. Summary of Tumor Data in Rats Fed Bladex®

	Males/Dose (ppm)					Females/Dose (ppm)				
	0	6	12	25	50	0	6	12	25	50
No. of animals examined[a]	62	19	23	23	30	63	21	23	23	32
Thyroid	(45)[b]			(19)	(24)	(47)				(26)
adenoma	4	4	7	2	9	7	6	6	6	7
carcinoma	5	4	2	3	1	8	1	0	3	3
Adrenal	(46)				(27)	(45)				(24)
adenoma	2	0	0	0	2	3	0	2	2	1
pheochromocytoma	4	7	6	8	5	4	0	0	1	2
Pancreas										
islet cell tumor	0	1	1	0	0	1	1	0	1	1
Kidney										
adenoma	0	0	0	0	1					
Mammary gland										
fibroadenoma						18	8	7	4	7
other tumors						0	1	1	0	1
Pituitary	(41)				(21)	(40)				(25)
adenoma	4	3	2	4	3	9	5	4	6	3
Other tumors	3	4	5	2	3	3	4	4	2	3

[a] The number of animals examined histologically is derived from Table 3 and corresponds to the data presented in the 1983 report.

[b] The number in parentheses are the actual number of tissues examined (compiled by this reviewer).

CONCLUSIONS:

This study is considered unacceptable for the assessment of the potential chronic toxicity and oncogenicity of Bladex in rats primarily due to numerous deficiencies resulting in a decreased capability of the test to detect possible compound-related effects. These deficiencies involved the following: the lack of overt toxicity in the dosed animals as compared to controls indicated that the animals could have tolerated higher dietary levels of the test material; limited number of tissues per animal were subjected to histologic examination; tumor incidences presented were not supported by the data and were calculated based on the number of animals tested rather than the number of specific tissues histologically examined; limited number of animals were available in the mid-dose groups for adequate evaluation; results of gross examination and histologic findings for non-neoplastic lesions were not adequately reported; clinical observations were not reported; and limited hematology, clinical blood chemistry, and urinalyses data were presented.

CORE CLASSIFICATION: Invalid.

DISCUSSION:

There are several major deficiencies present in this study which limits its value as an assessment of the chronic toxicity or oncogenic potential of Bladex® fed to rats. These deficiencies are discussed below:

1. Tumor data used for statistical analysis could not be verified for the following reasons:

   a. Several pathology sheets were illegible and thus limited the validation of tumors. For example, for control males and females 3 and 10 of the sheets, respectively were illegible, for 50 ppm females 3 pathology sheets were illegible.

   b. There were several pathology sheets in which corrections were made but these changes were not initialled or dated. Several tumors that were included in the author's tabulated presentation could not be verified. This was most noticeable for the diagnosis of tumors of the thyroids. This reviewer compared the histologic entries for thyroid on the pathology sheets against the tabulated diagnosis for all groups of males and females of the control and 50 ppm groups. Several discrepancies were noted. In addition, several of the entries for thyroid tumors did not specify whether the tumor was an adenoma or carcinoma. It was also noted that what was entered as hyperplasia in the original pathology sheet was often tabulated as adenoma.

   c. There was incomplete histologic examination of the animals. For many animals only major tissues were examined, rarely were there more than 8-12 tissue examined per animal. However, in the tabular presentation of tumor incidence data the number of animals examined histologically rather than the number of each target tissue examined histologically was used by the authors as the denominator. Table 5, prepared by this reviewer, summarizes the number of thyroids, adrenals, and pituitaries examined in control and 50 ppm animals.

TABLE 5. Tissues Examined Histologically

	Males		Females	
	0	50 (ppm)	0	50 (ppm)
No. of animals examined histologically	62	30	63	32
No. of thyroids	45	24	47	26
No. of adrenals	46	27	45	24
No. of pituitaries	41	21	40	25

2. It was not established that a maximum tolerated dose (MTD) was used in the study.

   a. There were no compound related toxic effects (e.g., body weight, clinical chemistry, hematology, organ weights or mortality).

   b. The weight-gain decrement in 25 and 50 ppm females and 50 ppm males compared to controls was transient. For example between weeks 0 and 24, 50 ppm females had a weight gain 108 g less than controls but in the interval between 24-52 weeks there was only an 11 g difference in weight gain (Table 2).

   c. A separate two-year feeding study in mice used levels up to 1000 ppm Bladex®.

3. The 1970 report stated that benign thyroid adenomas were lesions common of aging rats of the CFE strain (an inbred substrain of Sprague-Dawley rats) and that Haley (1978)[1] also noted a variable incidence of thyroid tumors ranging up to 50% in Sprague-Dawley rats. However, the historical tumor incidence provided information on "tumors" without further designation and without separating benign and malignant tumors of the adrenal or thyroid and therefore was not useful.

   Historical incidence of adrenal tumors subnitted on 7/12/84 classified adrenal medullary tumors. The range of pheochromocytomas in 5 studies was 6.3 to 18.9% (mean 14.3% for 286 animals). The current control incidence was 6.4%.

4. Non-neoplastic lesions could not be summarized because of illegible data. However, there was a high incidence of kidney lesions, mainly "chronic nephrosis," in all groups of animals even after week 44; this may indicate poor animal husbandry or that unthrifty animals were used in the study.

5. The study does not conform to guideline requirements for several other reasons:

   a. The number of animals in the mid dose groups was not sufficient for evaluation of oncogenicity.

   b. No clinical observations were provided.

   c. Gross lesions were not reported.

   d. Only major tissues were consistently examined histologically.

   e. There were inadequate clinical laboratory studies for a chronic toxicity study. Insufficient clinical chemistry tests were used, there use no differential leukocyte counts at the 44 week sacrifice, urinalysis data was incomplete and not tabulated in the report, and data at 18 months were only a few animals.

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EPA: 68-01-6561
TASK: 64
August 23, 1984

DATA EVALUATION RECORD

BLADEX^{R}

Oncogenicity/Chronic Oral Toxicity, Rat

VALIDATION OF DATA

APPENDIX

A. Check on number of tissues examined

CONTROLS MALES

	No. in group	No. of tissues examined		
		Thyroid	Adrenal	Pituitary
Terminal sacrifice	19	18	16	17
Deaths	23[a]	9	11	6
44-week sacrifice	18	13	14	13
18-mos. sac., deaths	6	15	5	5
TOTAL	66	45	46	41

[a] Pathology sheets illegible for males Nos. 10, 73, 109 (3)
No entries on histology sheet for males Nos. 15, 60, 87, 131 (4)
Autolysis - no entry for males Nos. 54, 65, 139 (3)

50 ppm MALES

	No. in group	No. of tissues examined		
		Thyroid	Adrenal	Pituitary
Terminal sacrifice	14	14	12	10
Deaths	7	3	5	4
44-week sacrifice	9	7	9	7
18-mos. sac., deaths	3	0	1	0
TOTAL	33	24	27	21

CONTROLS FEMALES

	No. in group	No. of tissues examined		
		Thyroid	Adrenal	Pituitary
Terminal sacrifice	20	20	19	16
Deaths	21[a]	6	6	1
44-week sacrifice	18	17	16	16
18-mos. sac., deaths	6	4	4	2
TOTAL	65	47	45	40

[a] Pathology sheets were illegible for animals No. 12, 21, 24, 103, 107, 120, 121, 131, 134, and 138.
Animal No. 18 was autolyzed and had no entries. No pathology sheets were found for animals No. 66, 67, 77.
Animal No. 66 was reported with thyroid adenoma and mammary adenoma; No. 77, thyroid carcinoma; No. 67, thyroid carcinoma and pituitary adenoma.

50 ppm FEMALES

	No. in group	No. of tissues examined		
		Thyroid	Adrenal	Pituitary
Terminal sacrifice	15	15	15	10
Deaths	6[a]	2	2	1
44-week sacrifice	9	7	6	7
18-mos. sac., deaths	3	2	3	3
TOTAL	33	26	24	25

[a] Pathology sheets were illegible for animals No. 64 124, 140, and 142. Female No. 142 was reported with thyroid adenoma and mammary tumor, No. 64, thyroid carcinoma and mammary tumor; No. 24, mammary tumor.

B. Animals with Questionable Diagnosis of Thyroid Tumors[a] (crossouts and corrections not initialed or dated)

	TABULATED	PATH SHEET ENTRY/COMMENT
Control Males		
# 5	med. carcinoma	diffuse hyperplasia of sheet cells "adenomatous" crossed out, "CA" added
# 17	adenoma	original entry written over in dark ink "small medullary T."
# 89	adenoma	foci of acinar cells, no tumor, "neoplasm" added and underlined
# 93	med. carcinoma	original entry written over in dark ink
#120	med. carcinoma	"medullary tumor" written over original entry
#124	adenoma	small focus hyperplasia = plus illegible entry
	45 thyroids were examined; 4 adenomas and 5 carcinomas were tabulated.	

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	TABULATED	ENTRY PATH SHEET/COMMENT
6 ppm Males		
# 12	adenoma	early proliferation?
# 51	med. carcinoma	"adenoma" crossed out, "solid sheets CA" added
# 55	carcinoma	diffuse tumor with some hyaline stroma no capsule but not unfiltrative
#128	adenoma	small focus, "hyperplasia" crossed out, adenomatous
	14 thyroids examined, 4 carcinomas and 4 adenomas were tabulated	
12 ppm Males		
# 58	adenoma	small medullary tumor
# 16	adenoma	small areas of adenomatous change
# 75	adenoma	"hyperplasia" crossed out, "adenoma" added
#105	bil. adenoma	(? verge malignant in one)
#112	adenoma	"little" hyperplasia crossed out, "adenoma" added
	med. carcinoma	massive adenoma, verge CA
#138	adenoma	"acute hyperplasia" crossed out, "adenoma" added
	17 thyroids were examined, 7 adenomas and 2 carcinomas were tabulated	
25 ppm Males		
# 78	med. carcinoma	"adenoma" crossed out, "CA" added
# 83	bilat. adenoma	written over it "medullary tumor"
#130	carcinoma	"adenoma" is original entry, "medullary tumor" written over entry
	19 thyroids examined, 2 adenomas, and 3 carcinomas tabulated	

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	TABULATED	ENTRY PATH SHEET/COMMENT
50 ppm Males		
# 50	adenoma	small focus of sheet prolif. med. tumor
# 72	adenoma	"early adenoma" crossed out and "medullary tumor" added
# 74	adenoma	adenoma "(? just hyperplasia)" crossed out, "medullary tumor" added
# 80	adenoma	minute focus of proliferation "adenoma" added in dark ink
#114	adenoma	entry "some hyperplasia??"

	TABULATED	ENTRY PATH SHEET/COMMENT
Control Females		
# 24	carcinoma	"adenoma" crossed out
#117	adenoma	medullary neoplasm
	47 thyroids were examined; 7 adenomas and 8 carcinomas were tabulated.	
6 ppm Females		
# 5	adenoma	bilateral medullary tumor
# 25	adenoma	small focus hyperplasia "(preneoplastic)" crossed out, "adenoma" added
# 68	adenoma	small focus "hyper plasia" crossed out, "adenoma" added
	pathology sheets #19 and 119 illegible	
12 ppm Females		
# 1	adenoma	small medullary tumor
# 33	adenoma	diffuse hyperplasia, "adenoma" added
	pathology sheets #144 and #71 illegible; #104 missing	

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25 ppm Females

# 2	adenoma	tiny nodule, "adenoma" added
# 78	adenoma	little medullary hyperplasia, "adenoma" added
	Pathology sheets #70 and #98 were illegible	

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50 ppm Females

#126	adenoma	medullary cell proliferation, "not yet tumor" was crossed out
	26 thyroids were examined; 7 adenomas and 3 carcinomas were tabulated	

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[a] According to NCI tumor nomenclature the synonym for C-cell adenoma is medullary adenoma (thyroid); C-cell carcinomas are medullary carcinomas (thyroid) and parafollicular carcinomas (thyroid). The term "medullary tumor" used often in this study should be classified as adenoma or carcinoma.

Kidney - nephrosis in control and 50 ppm males

	No. examined	No. nephrosis	Ave. grade
Control			
Terminal sacrifice	19	19	+3
18-mos sacrifice	5	5	+2
44-wk sacrifice	17	15	NG
50 ppm			
Terminal sac.	14	14	+1.68
18-mos sac.	-	-	-
44-wk sac.	9	9	NG