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

JUL - 1 1992

CASWELL FILE

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Bromacil - Review of a Combined Chronic/Oncogenicity Study in Rats for FIFRA 88 Phase V

Caswell No. 111
DP Barcode D164880

FROM: Elizabeth A. Doyle, Ph.D., Section Head
Review Section IV, Tox Branch II (H7509C) E A. Doyle 6/19/92

TO: Mario Fiol, PM 70
SRRD (H7508W)

THRU: Marcia van Gemert, Ph.D., Chief
Toxicology Branch II
Health Effects Division (H7509C) MvanGemert 6/23/92

Action Requested: Review of a combined chronic/oncogenicity study in rats (83-5) for reregistration phase V.

Study Summary: MRID No. 412617-01, Combined Chronic Toxicity/Oncogenicity Study with Bromacil: Two-year Feeding Study in Rats.

NOEL = 50 ppm (males - 1.96 mg/kg/day; females - 2.64 mg/kg/day)
LOEL = 250 ppm (males - 9.82 mg/kg/day; females - 13.3 mg/kg/day) based on decreased body weight gains in males and females during the second year of treatment.

No other significant treatment related effects were reported. No evidence of oncogenicity was reported.

Classification: Core - Guideline

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83-5
Combined Chonic/Oncogenicity in Rats
Dupont de Nemours, Wilmington, DE
August 16,1989
Study No. 186-89

Test material: Bromacil, 95% technical

Male and female Crl:CD(BR) rats were given bromacil in diet at 0, 50, 250, or 2500 ppm for two years.
NOEL = 50 ppm (males - 1.96 mg/kg/day; females - 2.64 mg/kg/day)
LOEL = 250 ppm (males - 9.82 mg/kg/day; females - 13.3 mg/kg/day) based on decreased body weight gains in males and females during the second year of treatment.

No other significant treatment related effects were reported. No evidence of oncogenicity was reported.

Classification: Core - Guideline

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DATA EVALUATION REPORT

BROMACIL

Study Type: Combined Chronic Toxicity/Oncogenicity in Rats

Prepared for:

Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by:

Clement International Corporation
9300 Lee Highway
Fairfax, VA 22031-1207

November 26, 1991

Principal Author: John Liccione Date 1/15/92
John Liccione

Reviewer: Wayne D. Reichardt Date 1-15-92
Wayne Reichardt

QA/QC Manager: Sharon A. Segal Date 1-15-92
Sharon Segal

Contract Number: 68D10075
Work Assignment Number: 1-18
Clement Number: 91-60
Project Officer: James E. Scott

EPA Review by: Elizabeth A. Doyle, Ph.D. Signature: E. A. Doyle
Review Section IV, Toxicology Branch I, Date: 6/19/92
Health Effects Division

Secondary EPA Review by: Elizabeth A. Doyle, Ph.D. Signature: E. A. Doyle
Section Head, Review Section IV, Date: 6/19/92
Toxicology Branch I, Health Effects Division

DATA EVALUATION REPORT

STUDY TYPE: **Guideline Series 83-5: Combined chronic toxicity/oncogenicity in rats.**

TEST MATERIAL: Bromacil

MRID Number: 412617-01

SYNONYMS: 5-Bromo-3-sec-butyl-6-methyluracil, IN N976

STUDY NUMBER: 186-89

SPONSOR: Agricultural Products Division, E.I. duPont de Nemours and Company, Inc., Wilmington, Delaware.

TESTING FACILITY: Agricultural Products Department, Experimental Station, Bldg. 402, E.I. duPont de Nemours and Company, Inc., Wilmington, Delaware 19898.

TITLE OF REPORT: Combined Chronic Toxicity/Oncogenicity Study with Bromacil: Two-year Feeding Study in Rats.

AUTHORS: Matthew S. Bogdanffy, Ph.D.

REPORT ISSUED: August 16, 1989

CONCLUSIONS: Bromacil was fed to male and female Crl:CD(BR) rats at dietary levels of 0, 50, 250, or 2500 ppm (males: 0, 1.96, 9.82, and 103 mg/kg/day; females: 0, 2.64, 13.3, and 144 mg/kg/day). Body weight gains in the high-dose males and females were significantly lower than those of the controls during the first and second years of treatment. The mid-dose males and females gained weight at a slower rate than the controls during the second year of treatment. Slight reductions in food consumption were noted in the high-dose males and females during the first and second years of treatment and in the mid-dose males and females during the second year of treatment. The reductions in food consumption in these animals were not proportional to the large decreases in body weight gains. Survival was increased in the high-dose rats of both sexes, and right be attributable to the decreased body weights of these animals. There was no significant effect of dosing on organ weights, ophthalmology, clinical signs, hematology, clinical chemistry (including

thyroid function), gross pathology, or histopathology. Bromacil was not oncogenic under the conditions of this study.

The LOEL is 250 ppm based on the decreases in body weight gains noted in males and females during the second year of treatment. The NOEL is 50 ppm.

CORE CLASSIFICATION: This study satisfies the Guideline requirements for a chronic toxicity/oncogenicity study (83-5) in rats and is classified Core Guideline.

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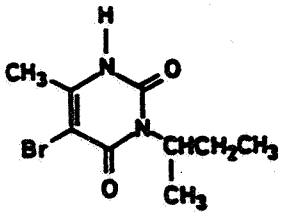
A. MATERIALS, METHODS, AND RESULTS

1. Test Article Description

Name: Bromacil

Formula:



Lot Number: 180-806 3T Batch 31, N.B. 5103-146

Purity: 95%

Physical Property: Colorless solid

Stability: Stable

2. Test Article Analyses for Purity and Stability

Test diets were prepared by adding an appropriate amount of bromacil to ground irradiated Purina Certified Rodent Chow #5002 and were thoroughly mixed to assure homogeneous distribution in the diet. All diets were prepared weekly.

The stability of the test substance in the diet (frozen immediately, 24 hours at room temperature, 10 days at room temperature, or 10 days refrigerated) was determined 1 day prior to study initiation (test day -1). The homogeneity of the test substance (samples derived from the top, middle, and bottom layers of the mixing vessel) and concentrations of the test substance in the diet were determined at days -1, 90, 181, 286, 293, 363, and 727.

Results of stability analysis indicated that the test substance, at concentrations of 50, 250, and 2500 ppm, was stable in diets freshly frozen, refrigerated for 10 days, or stored at room temperature for 24 hours or 10 days. Mean concentrations of bromacil in the diets at dose levels of 50, 250, and 2500 ppm were 99%, 97%, and 96% of target dose, respectively. Results of samples analyzed to determine

homogeneity of the test substance in the diet at levels of 50, 250, and 2500 ppm indicated a homogeneous mix.

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3. Animals

Rats (366 males and 363 females, Crl:CD BR strain) were received from Charles River Breeding Laboratories, Kingston, New York. Rats were acclimated to laboratory conditions for 10 days and were about 22 days of age upon arrival. During pretest, three rats/sex/cage (stainless steel, wire mesh) were housed in a room with temperature and humidity controls set at 23°C ± 2°C and 50% ± 10%, respectively, with a 12-hour light/dark cycle. After pretest, rats were housed individually. Water and food were provided ad libitum. One day after arrival, males weighed 39.7-69.0 g and females weighed 34.1-57.9 g.

Animals were assigned by sex to the following test groups using a computer-generated randomization procedure that stratified the animals within each sex by body weight:

Dietary Levels (ppm)	Main Study (24 months)		Interim Sacrifice (12 months)	
	Males	Females	Males	Females
0 (control)	62	62	10	10
50 (low)	62	62	10	10
250 (mid)	62	62	10	10
2500 (high)	62	62	10	10

Dose levels were selected on the basis of previous results of a 90-day oral study in rats and a 2-year chronic oral study in rats. These studies were not available for review.

4. Statistics

The Bartlett's test for homogeneity of variances was performed on the organ weight and clinical laboratory data. Body weight gains, organ weights, and clinical laboratory measurements were analyzed by a one-way analysis of variance. When the test for differences among test group means (F test) was significant, pairwise comparisons were made between test and control groups with the Dunnett's test.

Incidences of clinical observations were evaluated by the Fisher's Exact test with a Bonferroni correction and the Cochran-Armitage test for trend. Survival probabilities were estimated with the Kaplan-Meier procedure. Survival was evaluated with the Cochran-Armitage test for trend and the Fisher's Exact test with a Bonferroni correction.

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Tumor incidence was evaluated by the Fisher's Exact test and the Cochran-Armitage test for trend. Results of the pathological evaluation indicated that mortality-adjusted tests were not necessary. Significance was judged at the alpha = 0.05 level.

5. General Observations

(a) Mortality/morbidity/survival

Cage site examinations were conducted at least once daily throughout the study. The total scheduled (SD) and unscheduled (UD) deaths of animals of the main groups occurring during the study were reported as follows:

Group	Male		Female	
	SD	UD	SD	UD
0 ppm	21	41	22	40
50 ppm	18	44	28	34
250 ppm	17	45	28	34
2500 ppm	36*	26*a	37*	25*a

*Significantly different from control values ($p \leq 0.05$) by Fisher's Exact Test with a Bonferroni correction.

[a]Statistically significant trend by the Cochran-Armitage test at $p \leq 0.05$.

As shown by the data above, survival was significantly increased in the high-dose males and females of the main group. In the high-dose males, the increased survival was apparent beyond approximately day 469. In the high-dose females, the increased survival was evident at approximately day 567. Survival was 71% higher in the high-dose males and females when compared to controls at terminal sacrifice. The study author attributed the increased survival in the high-dose males and females to the decreased body weights in these animals. The increased survival was not regarded as an adverse effect. Mortality in affected rats was attributed by the study pathologist to pituitary adenomas and glomerulonephropathy, an age-related renal lesion. There were no compound-related effects on mortality in animals assigned to the interim group.

(b) Clinical observations

Rats were inspected at least once daily for signs of toxicity. The incidence of "teeth clipped" was significantly increased in the high-dose males (31 of 72 high-dose males versus 16 of 72 control males) and in the mid-dose females (25 of 73 mid-dose females versus 16 of 72 control females) and high-dose females

(31 of 72 high-dose females versus 16 of 72 control females). The incidences included rats assigned to the interim group. The incidence of "teeth clipped" was also significant ($p \leq 0.05$) by trend test. However, the study author did not consider this a toxicologically significant effect since it was probably related to decreased food consumption.

Increased incidences of masses of the mouth were seen in the high-dose males (7 out of 72 high-dose males versus 0 out of 72 control males) of both the main and interim groups. The increase in the masses was significant ($p < 0.005$) by Fisher's Exact test with a Bonferroni correction and was also significant ($p < 0.05$) by the Cochran-Armitage trend test. This clinical finding was not regarded by the study author to be of toxicological importance since there were no corresponding gross pathological or histopathological lesions.

(c) Body weights/food consumption/compound intake

Body weight. Individual body weights were recorded weekly during the first 6 months of the study and every 2 weeks thereafter.

Tables 1 and 2 summarize data on mean body weights and mean body weight gains, respectively, at selected intervals. Between weeks 1 and 51, mean body weights in the high-dose males were significantly ($p \leq 0.05$) lower (6.0-13.5%) than controls. Mean body weights in males receiving 50 and 250 ppm were comparable to controls during the first 51 weeks of treatment. Mean body weight in the high-dose males remained lower than controls from week 51 to week 103; mean body weight in the high-dose males was 17.8% lower than controls at the final weighing period (week 103). Mean body weight in males receiving 250 ppm were significantly lower (5.9 to 22.2%) than controls between week 61 and week 103. Mean body weights of low-dose males were significantly lower than controls only at week 103 (the final weighing period).

Between weeks 7 and 51, mean body weight in the high-dose females was significantly ($p \leq 0.05$) lower (5.0%-21.5%) than controls. Mean body weight in the high-dose females decreased to a maximimum of 32% at week 91. From week 93 to week 103, differences between body weights of high-dose females and controls tended to decrease as a result of decreasing mean body weights in the control animals. Mean body weight of the mid-dose females was significantly lower (10.3-12.4%) than controls between week 81 and week 103. Mean body weights of the low-dose females were consistently lower than controls between week 47 and week 97; however, the decrease in the mean body weights of these females was attributed by the study author to the increase in body weight gain in the control animals.

Reductions in mean body weight gain were also noted in males and females receiving 2500 ppm especially during the 3-6-month and 6-12-month intervals. Mean body weight gain of the high-dose males were significantly ($p \leq 0.05$) lower than controls by

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TABLE 1. Mean Body Weights (g ± SD) at Selected Intervals for Rats Fed Bomacil for 104 Weeks[a]

Weeks	Dietary Level (ppm) 0	50	250	2500
		Males		
0	140.4±11.1	140.7±11.2	140.8±11.1	141.1±113
13	545.2±47.5	539.9±51.7	533.7±47.1	497.9±43.0*
26	671.0±71.0	663.7±71.6	648.8±66.3	600.4±55.0*
51	796.5±114	773.9±101	762.4±86.5	688.9±72.3*
77	859.0±162	851.7±156	786.9±109*	754.1±100*
103	823.3±183	693.1±149*	640.2±157*	676.4±127*
		Females		
0	111.0±9.1	110.8±10.2	111.9±8.2	111.5±8.6
13	279.3±33.6	281.7±39.8	273.6±28.5	252.6±28.4*
26	332.5±53.7	335.7±61.2	318.7±45.4	282.6±38.5*
51	429.3±75.2	424.9±79.5	406.4±70.0	337.1±54.7*
77	548.8±116	519.0±123	501.8±100	401.7±75.6*
103	522.0±151	537.7±131	469.6±120	406.3±93.6*

[a]Data extracted from Tables 2 and 3 or the study report.

*Significantly different from control values, $p < 0.05$.

TABLE 2. Mean Body Weight Gains (g ± SD) at Selected Intervals for Rats Fed Bomacil for 104 Weeks[a]

Months	Dietary Level (ppm) 0	50	250	2500
		Males		
0-3	404.7±48.5	399.2±49.8	392.9±47.2	356.8±40.6*
3-6	125.8±37.3	123.7±32.6	115.4±31.3	102.5±26.1*
6-12	120.8±75.5	108.5±44.0	116.1±41.5	88.1±42.5*
0-12	656.2±116	633.1±101	621.7±86.4	547.8±70.6*
0-22	710.0±182	705.2±157	608.9±123*	563.6±128*
0-24	684.4±188	553.7±150*	501.0±158*	534.8±123*
		Females		
0-3	168.3±30.7	171.0±37.0	161.6±26.8	141.2±27.5*
3-6	53.2±28.8	54.0±28.4	45.1±26.4	30.0±16.5*
6-12	96.5±53.7	89.2±38.3	87.8±39.7	54.5±26.6*
0-12	318.3±72.4	314.1±75.7	294.5±68.0	225.6±54.5*
0-22	445.9±131	421.2±138	384.4±96.3*	293.6±94.1*
0-24	411.9±151	428.2±130	356.7±120	293.7±93.0*

[a]Data extracted from Tables 4 and 5 or the study report.

*Significantly different from control values, $p < 0.05$.

11.8%, 18.5%, 27.1%, and 16.5% during the 0-3-, 3-6-, 6-12-, and 0-12-month intervals. During these same time intervals, mean body weight gain of males fed 50 or 250 ppm were similar to controls. Mean body weight gain in males receiving 250 or 2500 ppm were significantly lower than controls by 14.2% and 20.6% between weeks 0 and 93.

Mean body weight gains of the high-dose females were significantly ($p \leq 0.05$) lower than controls by 16.1%, 43.6%, 43.3%, and 29.1% during the 0-3-, 3-6-, 6-12-, and 0-12-month intervals, respectively. Females fed diets containing 50 or 250 ppm exhibited body weight gains similar to controls during these same time periods. Mean body weight gain of females receiving 250 or 2500 ppm were significantly lower than controls by 15.7% and 35.6%, respectively, between week 0 and week 93. Mean body weight gain in the low-dose females were comparable to controls between week 0 and week 103.

Food consumption. Individual food consumption was determined weekly for the first 27 weeks and at 2-week intervals thereafter.

Table 3 summarizes selected data on mean food consumption. The food intake for the high-dose males was slightly lower than that of controls at the 0-3-, 3-6-, 6-12-, or 0-12-month intervals. The percent decreases at these monthly intervals were 7.4%, 6.8%, 6.1%, and 6.8%, respectively. During the 0-22- and 0-24-month intervals, food consumption in the high-dose males was lower by 6.0% and 6.3%, respectively; in the mid-dose males, food intake at these intervals was 3.0% and 4.1% lower than controls, respectively. Males fed 50 ppm diets displayed food consumption values similar to those of controls throughout the treatment period.

Mean food efficiency of the high-dose males was lower than controls by 4.8%, 11.5%, 23.0%, and 10.1% at the 0-3-, 3-6-, 6-12- and 0-12-month intervals. Food efficiency in the low- and mid-dose males was similar to controls when measured over the 0-12-month interval. Food efficiency was lower than controls by 17.1% during the 0-22- or 0-24-month intervals. Food efficiency in the mid-dose males was also lower than controls by 12.2% and 22.9% during these same time intervals. Food efficiency in the low-dose males was lower than controls by 17.1% during the 0-24-month interval.

The food intake of the high-dose females was lower than controls by 11.3%, 14.8%, 12.8%, and 13.2% during the 0-3-, 3-6-, 6-12- and 0-12-month intervals, respectively. During these same time intervals, food intake of the mid-dose females was reduced by 3.8%, 3.1%, 3.9%, and 3.6% when compared to controls. The slight decreases in food intake in the mid-dose females was not considered significant by the study author since the decreases were not accompanied by significant changes in body weight or body weight gain. Food consumption of the high-dose females was decreased by 11.3% and 9.8% at the 0-22- and 0-24-month

TABLE 3. Mean Food Consumption (g ± SD) at Selected Intervals for Rats Fed Bromacil for 104 Weeks[a]

Months	Dietary Level (ppm)			
	0	50	250	2500
		Males		
0-3	26.9	26.5	26.2	24.9
3-6	26.5	26.3	25.7	24.7
6-12	26.4	26.1	25.9	24.8
0-12	26.6	26.2	25.9	24.8
0-22	26.8	26.7	26.0	25.3
0-24	26.9	26.6	25.8	25.2
		Females		
0-3	18.6	18.8	17.9	16.5
3-6	19.6	19.8	19.0	16.7
6-12	20.3	20.1	19.5	17.7
0-12	19.7	19.7	19.0	17.1
0-22	20.4	20.3	19.8	18.1
0-24	20.4	20.4	19.8	18.4

[a]Data extracted from Tables 6 and 7 or the study report.

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intervals, respectively, when compared to controls. Food intake in the low-dose females was similar to controls at all sampling intervals.

Mean food efficiency of the high-dose females was decreased by 5.1%, 33.3%, 33.3%, and 17.8% compared to controls during the 0-3-, 3-6-, 6-12-, and 0-12-month intervals. Food efficiency in the mid-dose females was decreased by 0%, 13.3%, 3.7%, and 4.4% for the same intervals. The decrease in food efficiency in the mid-dose females at the 0-12-month interval was considered to be insignificant by the study author because body weights, body weight gains and food intakes in these females were similar to controls at the 0-12-month interval. Food efficiency in the high-dose females was decreased by 26.5% and 21.4% compared to controls at the 0-22- and 0-24-month intervals, respectively. During the 0-22- and 0-24-month intervals, food efficiency in the mid-dose females was decreased by 11.8% and 10.7%, respectively, in comparison with controls. Food efficiency in the low-dose females was decreased (compared to controls) by 7.4% at the 6-12-month interval and by 5.9% at the 0-22-month intervals, but was similar to control values at the 0-12- and 0-24-month intervals.

Compound intake. Mean compound intakes for males receiving 50 ppm, 250 ppm, or 2500 ppm at the 0-51 week interval were 2.29, 11.4, and 118 mg/kg body weight/day, respectively. In females receiving the same doses at this same interval, the compound intakes were 3.16, 15.8, and 159 mg/kg body weight/day, respectively.

Mean compound intakes for males receiving 50 ppm, 250 ppm, or 2500 ppm at the 0-103-week interval were 1.96, 9.82, and 103 mg/kg body weight/day, respectively. In females receiving the same dose at this time interval, the compound intakes were 2.64, 13.3, and 144 mg/kg body weight/day, respectively.

(d) Ophthalmoscopic examination

Ophthalmological examinations were performed on all rats prior to study initiation and on all surviving rats prior to study termination. At least 1 hour before each examination, one or two drops of 1% atropine sulfate solution were placed in each eye of every rat. Both eyes were examined by focal illumination and indirect ophthalmoscopy.

There were no statistically significant differences in ophthalmological findings between control and treated animals. However, pale ocular fundi were noted in several male and female rats. The incidence of pale ocular fundi in male rats receiving 0, 50, 250, or 2500 ppm was 1/28, 1/28, 1/23, and 3/44, respectively. In female rats this incidence was 1/30, 2/30, 1/33, and 4/43, respectively. Although the study pathologist indicated that this ocular finding could be related to anemia, no evidence of anemia was found following clinical evaluation. Several of the rats with pale ocular fundi also had retinal atrophy revealed by histopathological examination. When these

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rats were combined with those diagnosed clinically with linear focal retinopathy, the following incidences of potential retinal degeneration in males and females receiving 0, 50, 250, or 2500 ppm were noted: males-- 0/28, 0/28, 2/23, 4/44; females-- 1/30, 2/30, 1/33, 3/43. However, none of these incidences were statistically different from controls.

6. Clinical Pathology

Blood was collected from the orbital sinus of 20 rats/sex/per group (randomly selected) for clinical laboratory evaluations at approximately 3, 6, 12, 18, and 24 months. Blood samples from the first 10 rats/group were used for hematology and clinical chemistry evaluations. Serum samples from the remaining 10 rats/group were submitted for thyroid function analysis. Thyroid function tests were also performed after 9 months on test. Serum collected for thyroid function tests was assayed for thyroid stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4). Rats utilized for hematology and clinical chemistry evaluations were fasted for approximately 16 hours prior to blood collection. Rats designated for thyroid function assays were not fasted. Those parameters indicated by an X were examined:

(a) Hematology

X Hematocrit (HCT)*	X Leukocyte differential count
X Hemoglobin (HGB)*	X Mean corpuscular HGB (MCH)
X Leukocyte count (WBC)*	X Mean corpuscular HGB concentration (MCHC)
X Erythrocyte count (RBC)*	X Mean corpuscular volume (MCV)
X Platelet count*	Coagulation: thromboplastin time (PT)
Reticulocyte count (RETIC)	
Red cell morphology	

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with bromacil on hematology parameters were observed.

(b) Blood (clinical) chemistry

Electrolytes	Other
X Calcium*	X Albumin*
Chloride*	Albumin/globulin ratio
Magnesium*	X Blood creatinine*
Phosphorus*	X Blood urea nitrogen*
X Potassium*	X Cholesterol*
X Sodium*	X Globulins
	X Glucose*
Enzymes	Total bilirubin*
	Direct bilirubin
X Alkaline phosphatase (ALP)	X Total protein*
Cholinesterase	Triglycerides
Creatinine phosphokinase	
Lactic acid dehydrogenase	

X Serum alanine aminotransferase (SGPT)*
X Serum aspartate aminotransferase (SGOT)*
Gamma glutamyltransferase (GGT)

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with bromacil on clinical chemistry parameters were observed. Treatment with bromacil had no effect on thyroid function.

(c) Urinalysis

Urine samples were collected from the animals used for hematology and clinical chemistry investigations after 3, 6, 12, 18, and 24 months of treatment. Parameters indicated by an X were examined:

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

* = Recommended by Subdivision F (November 1984) Guidelines

No effects of treatment with bromacil were noted on urinalysis parameters.

7. Sacrifice and Pathology

All animals that were found dead, were sacrificed in extremis, or were sacrificed as scheduled received a complete gross examination. Tissues were fixed in neutral buffered formalin. Those tissues indicated by an X were collected for histopathological examination from all rats. Organs indicated by a double X (XX) were weighed:

Digestive System	Cardiovascular/Hematologic	Neurologic
Tongue	X Aorta*	XX Brain
X Salivary glands*	XX Heart*	X Peripheral nerve
X Esophagus*	X Bone marrow*	(sciatic nerve)*
X Stomach*	X Lymph nodes*	X Spinal cord
X Duodenum*	XX Spleen	(three levels)
X Jejunum*	X Thymus*	X Pituitary*
X Ileum*		X Eyes (Optic nerve)
X Cecum*	Urogenital	
X Colon*		
X Rectum	XX Kidneys*	Glandular
XX Liver*	X Urinary bladder*	
Gallbladder*	XX Testes*	X Adrenals*
X Pancreas*	X Epididymides	X Lacrimal gland
	X Prostate	X Mammary gland
Respiratory	X Seminal vesicle	XX Thyroids*
	XX Ovaries	XX Parathyroids*
X Trachea*	X Uterus	X Harderian glands
X Lung*		

Other

X Bone (sternum and femur)
X Skeletal muscle*
X Skin
X All gross lesions and masses

* = Recommended by Subdivision F (November 1984) Guidelines

Organs from rats found dead or sacrificed in extremis were not weighed. At both the 12- and 24-month sacrifices, the brain, heart, liver, spleen, kidney, testes, and ovaries were weighed wet. Adrenals, thyroid-parathyroids, and ovaries were weighed after fixation.

(a) Macroscopic

There were no compound-related gross observations among the animals sacrificed after 1 year (interim period).

A number of gross lesions, a number of which differed statistically from control incidences, were noted in animals sacrificed at termination (results summarized in Table 4). All of the lesions were incidental and/or age related, and not related to compound administration. In male rats, the findings included an increased incidence of tail nodules (associated with the tatooing procedure) and a decreased incidence of enlarged thyroids. In high-dose females, increased incidences of pituitary masses, mammary gland masses, brain compression, ovarian cysts, and teeth deformities were noted. The study author considered the pituitary masses and brain compression as possible contributing factors in the mortality of affected rats.

(b) Organ weights and body weight ratios

There were no organ weight changes of toxicological importance in animals sacrificed at interim or at termination.

Statistically significant changes in relative organ weights (for example: liver, kidney, spleen, and brain) noted in male and female rats sacrificed at interim were attributable to lowered body weights of the animals when compared to the controls. None of the absolute organ weight changes in the animals correlated with histopathological findings.

Although significant increases in mean absolute and relative brain weights were observed in treated males sacrificed at termination, the increases in the brain weights of these animals were attributable to the unusually low brain weights of several of the control males. Also, there were no corresponding histopathological findings in the brain.

TABLE 4. Representative Gross Lesions in Rats Fed Bromacil for 104 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)							
	Males				Females			
	0	50	250	2500	0	50	250	2500
Tail/nodule	10	9	11	21*	8	7	5	7
Parathyroid Glands/large	8	6	11	2*	1	1	1	0
Mammary Glands/mass	0	0	0	0	10	16	20*	28*
Pituitary/mass	21	24	26	15	35	35	38	48*
Brain/compression	19	25	23	15	37	35	38	51*
Ovaries/cyst	0	0	0	0	3	7	8	12*
Teeth/deformity	5	3	5	4	3	6	4	10*
Skin/alopecia	16	19	13	14	18	16	8*	4*

[a]Data extracted from Tables 36 and 37 of the study report.

[b]Based on 62 animals/sex/main group.

*Significantly different from control value, $p \leq 0.05$.

(c) Microscopic

Nonneoplastic. There were no biologically significant nonneoplastic findings in males and females at the 1-year interim sacrifice. The significant increase in the incidence of hemosiderin in the high-dose females (9 of 10 high-dose females versus 2 of 10 control females) was not supported by hematological findings.

Hepatic and retinal lesions were observed in animals at the 2-year terminal sacrifice (results presented in Table 5). A slight decrease in the incidence of hepatic periportal fatty change was noted in the high-dose males and females. The study author considered this finding to be a reflection of a physiologic adaptation to the lower body weight gain in the high-dose animals, and not an adverse effect. An increase in the incidence of age-related peripheral retinal atrophy (degeneration) was noted in the high-dose males and females. Peripheral retinal atrophy is distinguishable from toxic retinopathy. The increase in the incidence of retinal atrophy was not regarded by the study author to be treatment related. Other nonneoplastic findings noted in the study were considered to be unrelated to compound administration.

Neoplastic. There were no neoplastic lesions attributed to administration of bromacil.

The reviewer has no other comments regarding the materials and methods sections.

A description of the statistical analysis employed was included in the study report.

A Quality Assurance statement was included.

B. DISCUSSION

The study design was complete and adequate, and the data were well reported. A few summary pages in the front portion of the report (i.e., pages 15 and 17) were missing. A minor deficiency noted was that the adrenals, thyroid, parathyroid gland, and ovaries were weighed after fixation in buffered formalin. Dose levels were selected on the basis of the results of previous toxicity studies. Thyroid function tests were performed in this study since the results of a previous 2-year feeding study in rats suggested a possible compound-related effect on thyroid hyperplasia in rats recieving 2500 ppm.[1]

Because of the treatment-related decreases in mean body weights of mid- and high-dose males and females, as well as the treatment-related decreases in body weight gains, a maximum tolerated dose

TABLE 5. Representative Hepatic and Retinal Histological Lesions in Rats Fed Bromacil for 104 Weeks[a,b]

Organ/Finding	Dietary Level (ppm)									
	Males					Females				
	0	50	250	2500	0[c]	0	50	250	2500	0[c]
Liver										
Periportal fatty change	7	7	6	1*	-	12	4	7	1*	-
Eyes										
Retinal atrophy, diffuse, bilateral	1	0	0	0	2	7	6	5	12	6
Retinal atrophy diffuse, unilateral	0	0	1	0	0	2	2	2	3	3
Retinal atrophy focal, unilateral	0	1	2	2	1	1	1	1	1	1
Retinal atrophy peripheral, bilateral	1	1	3	3	6	7	11	6	19*	10
Retinal atrophy peripheral, unilateral	4	7	8	13*	7	13	10	15	9	11
Retinal atrophy, (Any type)	6	9	11	18*	16	30	29	27	40*	31
Retinal atrophy, peripheral (unilateral and bilateral)	5	8	11	16*	13	20	21	21	28	21

[a]Data extracted from Study Report, Pathology Report.

[b]Based on 62 animals/sex/main group.

[c]In order to clarify the background incidence of retinal lesions, the eyes from additional concurrent control male and female rats (derived from another chronic feeding study) were examined.

*Significantly different from control value, $p < 0.05$.

(MTD) was established. The reductions in body weights and body weight gains in the high-dose males and females were seen during the first and second years of the study. Decreases in body weights and body weight gains in the mid-dose males and females were noted during the second year of treatment. For the most part, the reductions in body weights and body weight gains in the mid- and high-dose males and females were accompanied by decreases in food consumption, although the reductions in body weights and body weight gains in these animals were disproportionate to the decreases in food consumption. Therefore, the reductions in body weights and body weight gains in these animals can only be partially explained by decreased food intake. Possible unpalatability of the mid- and high-concentration diets may have contributed to the decreased food intake, body weights, and body weight gains.

Mortality was significantly reduced in the high-dose rats and was attributed by the study author to the decreased food consumption in these animals. The mechanism by which reduced food intake increases survival is not certain. There were no toxicologically significant changes in hematology, clinical chemistry, thyroid function, organ weights, gross pathology or histopathology.

This reviewer agrees with the study author's conclusions that the no-observed-effect level for chronic effects at 1 year was 250 ppm, and at 2 years was 50 ppm, and that there was no oncogenic effect under the conditions of this study.

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[1]Haskell Laboratory Report No. HLR-21-66. Long-term feeding tests with 5-bromo-3-sec-butyl-6-methyluracil (bromacil).