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CASWELL FILE

125B

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

005834

APR 15 1987 ~~APR 14 1987~~

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Registration No. 40810-6 - TB Proj. No. 1457
Terbuthylazine - Chronic Toxicity/Oncogenicity Studies (2), Rat (Ciba-Geigy Nos. 785196 and 791229); and Chronic Toxicity/Oncogenicity Study, Mouse (Ciba-Geigy No. 785195).

Tox. Chem. No.: 125B

FROM: David G. Van Ormer, Ph.D. DVO 2 Apr 87
Section III, Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: John H. Lee, PM 31
Disinfectants Branch
Registration Division (TS-767C)

THRU: Marcia van Gemert, Ph.D. Marcia van Gemert 4/3/87
Head, Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

and

Theodore M. Farber, Ph.D. 4/14/87
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

Note to Registration Division

In order for TB to complete the assessment of mammary tumors in Study No. 785196, a Peer Review will be conducted.

1

To aid in the assessment we are requesting the registrant to provide information as follows:

1. Appropriate historical control data for mammary tumors in the same rat (with respect to strain, stock, and laboratory) as was utilized in Study Nos. 785196 and 791229.

2. A tabulation of the "Number of Tissues Examined" for Study No. 785196 with respect to:

   a. Female mammary gland.

   b. Testis.

The above requests were transmitted verbally to John Lee (557-0485, 7470) on February 13, 1987.

We are also requesting that comments on the studies of this action be withheld until after the Peer Review. At that time we will return the data. Toxicology Branch will also provide amendments (if needed) to Study Nos. 785196 and 791229.

Brief Summaries of Subject Studies

1. Study No. 785196 (chronic toxicity/oncogenicity, rat)

   Rats of the strain Tif:RAIf received Terbuthylazine in the diet at concentrations of 0, 30, 150, or 750 ppm, administered to dosage groups of 80 rats per sex. After treatment for 104 weeks, the animals were placed on untreated diets until final sacrifice at week 112 for males and week 122 for females. Thus, the recovery period was up to 8 weeks in males and 18 weeks in females.

   No clinical signs.
   Dose-dependent decrease in body weights and feed consumption, both sexes.
   Dose-dependent increase in survival time, both sexes.
   Decreased RBC count, hemoglobin concentration, and hematocrit in high-dose females at more than one test period. Prothrombin time prolonged in males at top dose.
   Decreased urine volume at top dose, both sexes (at more than one period).
   Dose-dependent liver cysts in both sexes, except low-dose males.

Nodular hyperplasia of the thyroid in high-dose females.
Nodular hyperplasia of Leydig cells at high dose.
Increased mammary carcinomas in low- and high-dose females ($p < 0.05$).
Increased benign interstitial cell tumors in high-dose males ($p < 0.05$).
Increased C cell adenoma in males at mid and top dose (not statistically significant).

NOEL = Less than 30 ppm (LDT) (decreased body weights and feed consumption, dose-related in both sexes).

Oncogenic potential: increased mammary carcinoma, females at low and top dose (30 and 750 ppm).

Core Classification: Supplementary.

2. Study No. 791229 (chronic toxicity/oncogenicity, rat)

Rats of the strain Tif:RAIf received Terbuthylazine in the diet at concentrations of 0, 6, or 30 ppm, administered to dosage groups of 80 rats per sex. After treatment for 98 weeks, the animals were placed on untreated diets until final sacrifice at week 116 for males and week 121 for females. Thus, the recovery period was up to 18 weeks in males and 23 weeks in females.

Same strain of rat (Tif:RAIf) as in Study No. 785196.

Decreased body weights and feed consumption at 30 ppm (HDT).

No other toxicity parameters showed compound-related effects.
Tumor incidences not elevated at either dose, 6 or 30 ppm.

NOEL = 6 ppm (LDT)
LEL = 30 ppm (HDT): decreased body weights and feed consumption.

Core Classification: Minimum.

3. Study No. 785195 (chronic toxicity/oncogenicity, mouse)

Mice of the strain Tif:MAGf(SPF), 50 animals per sex per dosage group, received Terbuthylazine in the

diet at the respective concentrations of either 0, 30, 150, or 750 ppm. Terminal sacrifice occurred at the end of the 24-month treatment.
No clinical signs.
Survival at top dose was 50 percent or greater.

Female body weight significantly depressed at top dose.
Feed consumption somewhat depressed at top dose.

Sodium and urea notably reduced in males.
Urine volumes decreased at all doses, both sexes.

Thyroid and adrenal weight and O/BW ratios elevated in females, with some dose relationship seen.
Brain weights trend larger toward top dose, both sexes.
Spleens enlarged in females.
Thyroid follicles dilated in males at top dose.

Other microscopic findings without apparent treatment effect.

Possible antithyroid effect.

Elevated thyroid weights and ratios, females (some dose relationship).
Dilated thyroid follicles, males (top dose).

Primary tumor incidences showed no treatment-related effects.

NOEL not observed at low dose for several parameters.

LEL = 30 mg/kg/day (LDT)

Effects at low dose:

Males - reduced sodium, urea, and urine volume.

Females - elevated thyroid weights and ratios with significant positive trend in O/BW ratios.

Core Classification - Minimum.

CASWELL FILE

Reviewed by: David G. Van Ormer, Ph.D. DVO 2 Apr 87
Section III, Toxicology Branch (TS-769C)
Secondary reviewer: Marcia van Gemert, Ph.D.
Section III, Toxicology Branch (TS-769C)

DATA EVALUATION REPORT

Study Type: Chronic Feeding/Oncogenicity - Mouse

Tox. Chem. No.: 125B

Accession Number: 261458

MRID No.: N/A

Test Material: Terbuthylazine

Synonyms: GS 13529 Technical, Belclene® 329

Study Number: 785195 (GU)

Sponsor: Ciba-Geigy, Ltd.

Testing Facility: Ciba-Geigy, Ltd.

Title of Report: Chronic Toxicity and Carcinogenicity Study in Mice

Author: W. Gfeller, Study Director

Report Issued: August 1982

Conclusions:

Dose groups of 50 animals per sex received either 0, 30, 150 or 750 ppm test material in the diet for 24 months.

Signs of toxicity not observed. Survival at top dose was 50 percent or greater. At top dose, the female weight was significantly depressed from Week 9 to 101. Feed consumption was somewhat depressed at top dose, particularly in males.

In males (but not in females), there were dose-related increases in erythrocytes, hemoglobin, and hematocrit, with statistically significant ($p \leq 0.05$) elevations at mid and top dose. Lymphocytes showed a negative dose response, with a significant depression ($p \leq 0.05$) at top dose. Males also showed reductions in sodium and urea, with significant depressions at top dose (and also for sodium at low dose; $p = 0.05$). Females presented a dose-related decrease in cholesterol, with significantly depressed values ($p \leq 0.05$) at both mid and top dose. Urine volumes were significantly decreased at all doses in both sexes ($p \leq 0.05$), except for females at mid dose.

In females, the thyroid absolute weights and their O/BW ratios and O/brain weight ratios are notably elevated. Thus, the thyroid/body weight ratios are significantly ($p \leq 0.05$) elevated at each dose, and there is a positive dose-response trend, according to the Report. Male thyroid weights and ratios, on the other hand, are decreased from control, with both ratios significantly depressed ($p \leq 0.05$) at mid and top dose. Female brain and adrenal O/BW ratios show positive dose responses, with significant elevations at top dose ($p \leq 0.05$). Female gonad weight and both the O/BW and O/brain weight ratios are depressed significantly at top dose ($p \leq 0.05$), with significant negative trends in both the absolute weights and the O/brain weight ratios.

Macroscopic examination revealed enlarged spleens (females only), and dilatation of the uterus and ovarian cysts, all without dose relation.

Microscopical findings were without apparent treatment relation.

Data on primary tumors showed no treatment-related effects. Various organs in all dose groups, including control, exhibited malignant lymphoma, no dose-related.

Some of the clinical and organ weight findings of this study appeared somewhat contradictory or at least not mutually supporting. In addition to the possibility of a difference in response between the sexes, the difficulty of weighing (without bias) the small, wet organs of the mouse may account for some of this lack of consonance.

NOEL Summary:

NOEL not observed at low dose for several parameters.

LEL = 30 ~~mg/kg/day~~ ppm (LDT).

Effects at Low Dose:

Males

Sodium and urea are notably reduced at all doses, with marked reductions at low dose. Urine volumes are significantly decreased in both sexes at all doses except mid-dose females.

Females

Thyroid weights and O/BW ratios significantly elevated at low dose, with a statistically significant positive dose-related trend in the ratios.

Classification:

Minimum Data (no eye examinations; hematological/clinical findings reported only for termination).

A. Materials:

1. Test compound: GS-13529; Description: white powder; Batch #590305; Purity 98%, contaminants: results available from Ciba-Geigy.

2. Test animals: Species: mouse; Strain: Tif:MAGf (SPF), meaning F_3 hybrid of inbred NMRI = MAG (Tif) x NIH/NMRI Tif; Age: 4-5 weeks; Weight: males 27.5 g, females 23.5 g; Source: Ciba-Geigy Switzerland.

B. Study Design:

1. Animal assignment - Animals were assigned randomly to the following test groups (Interim sacrifice was not performed):

Test Group	Dose in Diet (ppm)	Main Study 24 Months Male	Female
1. Control	0	50	50
2. Low (LDT)	30	50	50
3. Mid (MDT)	150	50	50
4. High (HDT)	750	50	50

2. Diet preparation - Diet was prepared at unstated periods and stored at unstated temperature. Samples of treated food were analyzed for stability and concentration at Ciba-Geigy.

Results - Available from Ciba-Geigy.

Water analysis is performed by local Swiss and Ciba-Geigy laboratories, from which results are available.

3. Animals received food and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data: Nonparametric methods for comparison of treated group with control. Trend was measured according to Jonckheere. Survival analysis was by the generalized Wilcoxon Test and the generalized Savage Test.

7

5. Quality assurance was not verified periodically. The final report was audited on August 27, 1982. The QA Manager was L. Durand.

C. Methods and Results:

1. Observations - Animals were inspected daily for signs of toxicity and mortality.

Toxicity/Mortality (survival)--The Report states that no signs of toxicity were observed in the treatment groups.

Mean survival in each male treatment group was greater than in control:

Dose (ppm)	Median Survival (days) Male	Female
0	651	723
30	740	707
150	710	681
750	> 743	720

Among males, 50 percent survived to terminal sacrifice at top dose. For females, the survival differences between treated and control were much less marked.

2. Body weight - Animals were weighed weekly for the first 3 months, and at least monthly thereafter. Males at top dose showed somewhat depressed weight (relative to controls) at all periods between Week 11 and 80. Females showed a dose-related increase in weight depression ($p \leq 0.01$) from Week 10 to Week 101. The top dose in females was significantly depressed ($p \leq 0.01$) from Week 9 to 101.

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.

Mean food consumption in males shows a dose-related decrease essentially between Weeks 5 and 80. Statistically depressed consumption ($p \leq 0.01$) extended from Week 5 to Week 54. For females, the significant trends and depressions in food consumption were much more scattered and occurred before Week 50.

Food conversion (efficiency) was calculated as:

$$\frac{\text{Weekly food consumtion}}{\text{Midweek body weight}} \times \frac{1000}{7}$$

8

For males, the mean food conversion of treated groups generally lie somewhat below controls. With females, however, the reverse is true, with food conversion of treated groups somewhat above that of controls.

Mean water consumption of treated males was somewhat below the water consumption of controls through Week 95. Female water consumption was unremarkable.

Mean compound intake, as calculated from food conversions and analyses of test in material in the diet (at 15 periods) is tabulated as follows:

	Males	Females
	mg/kg/day	mg/kg/day
Low	3.28	3.22
Mid	16.99	16.66
High	86.76	88.54

4. Ophthalmological examinations - Hearing tests and eye examinations were not performed, contrary to protocol indications.

5. Hematology - Blood was collected before treatment and at 104 weeks for hematology and clinical analysis from 12 mice of each sex and group randomly selected from survivors. The CHECKED (X) parameters were examined.

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

In males, there were dose-related increases in erythrocytes, hemoglobin, and hematocrit. These increases were significant ($p < 0.05$, one-sided Dunnett t-test by reviewer) at the mid and top doses. There is a dose-related decrease in lymphocytes in males, with significance at top dose ($p < 0.05$, one-sided Dunnett t-test by reviewer).

Hematological Alterations in Males
(mean values)

	Control	Low	Mid	High
RBC's (10^6/cc)	8.0	8.6	9.7[a]/	10.7[a]/
Hemoglobin (mmol/L)	6.4	7.0	8.1[a]/	8.4[a]/
Hematocrit (%)	36.0	38.0	43.0[a]/	44.0[a]/
Lymphocytes (G/L)	3.7	3.5	2.6	1.9[a]/

[a]/ $p < 0.05$, one-sided Dunnett t-test by reviewer.

6. Clinical chemistry

X

Electrolytes:

[ ] Calcium*
[ ] Chloride*
[ ] Magnesium*
[ ] Phosphorous*
[X] Potassium*
[X] Sodium*

Enzymes

[X] Alkaline phosphatase
[ ] Cholinesterase
[ ] Creatinine phosphokinase*
[ ] Lactic Acid dehydrogenase
[X] Serum alanine aminotransferase (also SGPT)*
[X] Serum aspartate aminotransferase (also SGOT)*

X

Other:

[ ] Albumin*
[ ] Blood creatinine*
[ ] Blood urea nitrogen*
[X] Cholesterol*
[ ] Globulins
[X] Glucose*
[ ] Total Bilirubin*
[X] Total Protein*
[ ] Triglycerides
[X] Urea

In males, sodium is decreased at all doses, significantly ($p \leq 0.05$) at low and high dose. Urea shows a significantly ($p \leq 0.01$) dose-related decreasing trend, with a 35 percent reduction (relative to control) at low dose.

Females show a dose-related decrease in cholesterol.

7. Urinalysis - Pooled urine was collected from five randomly selected animals per sex per group at Week 104. Prior to and during urine collection, the animals were fasted overnight in special metabolism cages. The CHECKED (X) parameters were examined.

X

[ ] Appearance*
[X] Volume*
[X] Specific gravity*
[X] pH
[X] Sediment (microscopic)*
[X] Protein*

X

[X] Glucose*
[X] Ketones*
[X] Bilirubin*
[X] Blood*
[ ] Nitrate
[ ] Urobilinogen

Urine volume in males shows a significantly ($p \leq 0.01$) dose-related decrease, with significantly ($p \leq 0.05$) reduced volumes at mid and top dose.

Clinical Chemistry Alterations
(mean values)

	Control	Low	Mid	High
Males				
sodium (mmol/L)	161	153[b]/	156	152[b]/
urea (mmol/L)	19.4	12.5	10.8	6.1[b]/
Females				
cholesterol (mmol/L)	2.49	2.22	1.89[a]/	1.52[a]/

Urine Volumes
(mean values)

	Control	Low	Mid	High
Males (mL)	2.30	1.44[a]/	0.83[a]/	0.91[a]/
Females (mL)	1.85	1.05[a]/	1.75	1.55[a]/

[a]/ $p < 0.05$, one-sided Dunnett t-test by reviewer.
[b]/ $p = 0.05$, according to Report.

In females, the urine volumes are also decreased at all doses, particularly at low dose, where specific gravity is significantly ($p \leq 0.05$) elevated in both sexes.

Summary tables for urinalysis include only the parameters volume, specific gravity, and pH.

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

Digestive system	Cardiovasc./Hemat.	Neurologic
[ ] Tongue	[X] Aorta*	X[X] Brain* (cerebrum, cerebellum, brainstem)
[X] Salivary glands*	X[X] Heart*	[X] Sciatic nerve*
[X] Esophagus*	[X] Bone marrow*	[X] Spinal cord (3 levels* not indicated)
[X] Stomach*	[X] Lymph nodes* (axillary mesenteric and cervical)	
[X] Small intestine		
[ ] Duodenum		
[ ] Jejunum*		

11

Digestive system	Cardiovasc./Hemat.	Neurologic
[ ] Ileum*	X[X] Spleen*	X[X] Pituitary*
[X] Large intestine	X[X] Thymus*	[X] Eyes (optic n. not taken)
[ ] Cecum*	Urogenital	Glandular
[ ] Colon*	X[X] Kidneys*	X[X] Adrenals*
[ ] Rectum*	[X] Urinary bladder*	[X] Lacrimal gland and Harderian gland
X[X] Liver*	[X] Testes*	[X] Mammary gland*
[X] Gallbladder*	[X] Epididymides	[ ] Parathyroids*
[X] Pancreas*	[X] Prostate	X[X] Thyroids*
[X] Peritoneum	[X] Seminal vesicle	Other
Respiratory	[X] Ovaries	[X] Bone* (with marrow)
[X] Trachea*	[X] Uterus*	[X] Skeletal muscle*
X[X] Lung*		[X] Skin
		[X] All gross lesions and masses

a. Organ weight and organ/body weight (O/BW) ratios

Males -

The mean heart weights and O/BW ratios at all doses are somewhat elevated over control values. Thyroid O/BW ratios at mid and top doses are significantly ($p \leq 0.05$) reduced, compared to controls, according to the Report. The Report notes a significant elevation in brain weight at top dose in males.

The tabulated organ-to-brain weight ratios provide no additional information on the males.

Females -

The brain/body weight ratio is elevated at top dose ($p \leq 0.05$) relative to control values, and the ratio has a statistically significant positive trend ($p \leq 0.01$). Both the absolute adrenal weights and their O/BW and O/brain weight ratios are significantly ($p \leq 0.05$) elevated at top dose, and each parameter shows a significant ($p \leq 0.01$) positive trend. At the mid and top dose, the female gonads (absolute weights, O/BW ratios, and O/brain weight ratios) show a negative trend, with all three parameters significantly ($p \leq 0.05$) reduced at top dose, relative to controls.

In females, again, the thyroid absolute weights, O/BW ratios, and O/brain weight ratios are all significantly elevated at low and top dose ($p \leq 0.05$, according to

the Report). In fact, the thyroid O/BW ratios are significantly elevated at all three doses, and exhibit a positive dose-response trend, according to the Report.

Mean Organ/Body Weight Ratios and
(Mean Organ/Brain Weight Ratios)

	Control	Low	Mid	High
Males				
Heart	0.440	0.514	0.532	0.482
Thyroid	0.054 (4.759)	0.055 (4.538)	0.039[a]/ (3.283)[a]/	0.044[a]/ (3.780)[a]/
Females				
Brain	1.224	1.273	1.302	1.426[a]/
Adrenals	0.0452 (3.651)	0.0494 (3.953)	0.0560 (4.285)	0.0634[a]/ (4.495)[a]/
Gonads	0.200 (15.744)	0.407 (30.166)	0.128 (10.488)	0.104[a]/ (7.345)[a]/
Thyroid	0.0477 (3.959)	0.0662[a]/ (5.330)[a]/	0.0619[a]/ (4.821)	0.0715[a]/ (5.081)[a]/

[a]/ $p \leq 0.05$, according to Report.

b. Gross pathology

Macroscopic examination shows no treatment-related effects. In males there are frequent individual tabulations of perineal cysts and enlarged (inflamed) seminal vesicles at all doses, including control. The Report notes that masses in the chest wall were more frequent in control males than in treated males: 13 vs. 4, 5, and 3, respectively, in the three ascending dose groups, each containing 50 males.

Particularly in females, the spleen is tabulated as large, an effect without dose relation. The females also show dilation of the uterus; ovarian cysts; and fluid in the uterine horns and the abdominal and thoracic cavities. These effects are all without dose relation.

Masses appear to be accompanied by microscopic findings.

c. Microscopic pathology

1. Non-neoplastic

The summary table of microscopical findings shows no tabulations of treatment-related significance.

The adrenals exhibit ceroid deposition in all dose groups (including controls), particularly in females. The adrenal cortex presents subcapsular proliferation, again in all dose groups including controls, and particularly in females. The lungs show lymphocytic infiltration with somewhat higher incidence in treated groups than in control, but there is no dose relation. Gastric mucosa contains hyperplasia at all doses (including controls), and is not dose-related. Lymphocytic infiltration of the kidney and urinary bladder is prevalent in all groups (including controls), particularly in males.

The spleens of control males show extramedullary hematopoiesis and amyloidosis, with larger incidence than in any of the other sex-dose groups. Five males at top dose showed dilation of the thyroid follicle (zero in controls). Dilatation and chronic inflammation of the seminal vesicles occurs at all doses (including controls), and is not dose-related. Pressure atrophy occurs at the brain base in eight animals, is not dose-related, and does not occur in controls.

2. Neoplastic

The summary table of primary tumors shows no treatment-related effects. Various organs (viz., spleen, lymphoreticular tissue, lymph node [unspecified], lungs, salivary gland, liver, pancreas, stomach, kidneys, urinary bladder, adrenals, mediastinum, and peritoneum) show a prevalence of malignant lymphoma (systemic infiltration) in all dose groups, including control. The effect occurs particularly in the females, and has no dose relation. Among control males, the incidence of fibrosarcoma in subcutaneous tissue is somewhat elevated above each of the three incidences for the male treatment groups.

D. Discussion

A possible antithyroid effect is given partial evidence by the elevated thyroid weights and ratios in females, and by the dilation of thyroid follicles in males at top dose.

Comparison of effects with those of other studies:

Brain: Elevated weights in males (top dose), and elevated body weight ratios in females (top dose) reflect the elevated weights in males at all doses in the chronic feeding study, rat, 1-year report (C.G. No. 785196); and also the elevated ratios in females at both doses in the 3-generation reproduction study (IBT No. B8272, Valid). Also, these reflect the brain/body weight increases (both sexes) of the 28-day dermal toxicity study (C.G. No. 8206300).

Adrenals: Elevated weights and ratios (top-dose females) reflect the elevated ratios in males at both doses tested in the chronic feeding study, rat, 1-year report (C.G. No. 791229), and also reflect the elevated adrenal weights of males in the 28-day dermal toxicity study, rabbit (C.G. No. 8206300).

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

CASWELL FILE

005834

EPA: 68-02-4225
DYNAMAC No. 2398
January 15, 1987

DATA EVALUATION RECORD

TERBUTHYLAZINE (BELCLENE)

Chronic Toxicity/Oncogenicity Study in Rat

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-15-87

CASWELL FILE

EPA: 68-02-4225
DYNAMAC No. 239-B
January 15, 1987

DATA EVALUATION RECORD

TERBUTHYLAZINE (BELCLENE)

Chronic Toxicity/Oncogenicity Study in Rats

REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: January 15, 1987

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 1-15-87

APPROVED BY:

I. Cecil Felkner, Ph.D.
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-15-87

David Van Ormer, Ph.D.
EPA Reviewer

Signature: D.L. Van Ormer
Date: Feb 9, 1987

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: Feb 10, 1987

DATA EVALUATION REPORT

TOX. CHEM. NO.: 125B
MRID NO.: N/A

STUDY TYPE: Chronic toxicity/oncogenicity study in rats.

ACCESSION NUMBER: 261870-261871.

TEST MATERIAL: GS 13529.

SYNONYMS: Terbuthylazine.

STUDY NUMBER(S): 791229.

SPONSOR: CIBA-GEIGY Ltd., Basle, Switzerland.

TESTING FACILITY: Sisseln facility, 4332 Stein, Switzerland.

TITLE OF REPORT: Lifetime carcinogenicity and chronic toxicity study in rats.

AUTHOR(S): Gfeller, W.; Basler, W.; Zak, F.; Hess R.

REPORT ISSUED: June 17, 1983.

CONCLUSIONS:

Terbuthylazine was administered to male and female rats (Tif:RAIF) in the diet at concentrations of 0, 6, or 30 ppm for 98 weeks; thereafter, animals were placed on diets lacking the test material until final sacrifice at week 116 for males and week 121 for females. Thus, the recovery period was up to 18 weeks in males and 23 weeks in females.

Under the conditions of the study, terbuthylazine was not oncogenic in male or female rats (Tif:RAIf) receiving 6 or 30 ppm in the diet. There was a compound-related decrease in body weights and food consumption during the first 26 weeks of the study in males receiving 30 ppm, whereas females receiving 30 ppm had lower body weights throughout the dosing period. There were no effects on survival, hematology, clinical chemistry, urinalysis, mean organ weights, gross lesions, and histopathology. The NOEL and LOEL for chronic toxicity are 6 and 30 ppm, respectively, of terbuthylazine in the diet. This is in agreement with a previous study (report No. 785196) conducted with dietary levels of 30, 150, and 750 ppm, in which similar effects were seen at 30 ppm (LDT).

The basis for MTD is the data of study No. 785196 showing significantly decreased weight at 90 days in both sexes for all dose groups, including 30 ppm.

Classification: Core Minimum when considered together with body weight data of study No. 785196.

A. MATERIALS:

1. Test Compound: GS 13529; description: white powder; batch No. 590305; purity: 98%; contaminants: not reported.

2. Test Animals: Species: rat; strain: Tif:RAIf (SPF); age: 3 weeks; weight: males--74-75 g, females--72-73 g; source: Animal Production, CIBA-GEIGY, Ltd.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (27 Months)		Interim Sacrifice (12 and 24 Months)	
		Males	Females	Males	Females
1 Control	0	80	80	10	10
2 Low (LDT)	6	80	80	10	10
3 High (HDT)	30	80	80	10	10

The animals were acclimated to laboratory conditions for 1 week prior to treatment. Rats were housed in groups of five per cage and placed on treated diet for 98 weeks only; thereafter, the animals received untreated diets. Final sacrifice of males and females was at weeks 116 and 121, respectively.

2. Diet Preparation: Diet was prepared at unspecified periods and stored at an unspecified temperature. Samples of food containing test material were analyzed for stability and concentration at weeks 4, 12, 18, 28, 38, 48, 59, 68, 77, 87, and 98.

   Results: Analysis of the test material showed that mean percent concentrations were 95.7 and 95 percent for the 6- and 30-ppm test diets, respectively.

3. Animals received certified standard diet (Nafag) and water ad libitum.

4. Statistics: For each time interval and parameter, a univariate statistical analysis was conducted. Each treated group was compared to the control group with respect to dispersion and displacement. In addition, a trend test was applied that covered all groups. Survival analysis was performed by the generalized Wilcoxon test and the generalized Savage test. The incidence of histopathological lesions was investigated using a method described by R. Peto et al.

5. Quality assurance was dated July 13, 1983.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of toxicity and mortality.

   Results: Toxicity--No signs of toxicity were observed. Eye opacity and hair loss were observed frequently but the incidences were similar in control and dosed groups. However, the incidence of eye opacity increased in females receiving 30 ppm between month 21 and study termination.

   Mortality (survival)--Survival in dosed animals was similar to that of controls throughout the study (Table 1). Final sacrifice for males and females was on weeks 115 and 119, respectively, of the study.

2. Body Weight: Rats were weighed weekly for 3 months and monthly thereafter.

   Results: The mean body weight of males in the low-dose group was similar to control animals throughout the study. In high-dose males, mean body weights were consistently lower than control animals; the differences were statistically significant during weeks 2 to 26 of the study (Table 2). Mean body weights of

TABLE 1. Survival of Rats Fed Diets Containing GS 13529 for 98 Weeks

Test Group	No. of Live Animals at Week[a]				
	0	52	80	105	116/121[b]
Males					
Control	80	80	59	32	10
6 ppm	80	79	58	28	8
30 ppm	80	79	60	29	11
Females					
Control	80	79	66	40	16
6 ppm	80	79	62	45	13
30 ppm	80	80	63	45	224

[a]Ten animals from each group were killed at interim sacrifice (weeks 52 and 104 of the study).

[b]Males were sacrificed in week 116 and females in week 121 of the study; values calculated by our reviewers.

TABLE 2. Mean Body Weights of Rats Fed Diets Containing GS 13529 for 98 Weeks

Test Group	Mean Body Weights (g) at Week						
	-1	13	26	52	80	105	115/119[a]
Males							
Control	75	503	643	794	883	813	599
6 ppm	74	500	635	786	865	781	698
30 ppm	75	475*	605*	759	857	782	637
Females							
Control	72	304	369	445	552	588	507
6 ppm	73	304	364	442	533	537	478
30 ppm	72	293	347*	413*	492*	520	494

[a]Males were last weighed during week 115 and females during week 119 of the study.

*Significantly different from control value ($p < 0.05$).

females in the high-dose group were similar to control animals during the first 18 weeks of the study. Thereafter, the mean body weights were significantly lower than control values. In the low-dose animals, mean body weights were similar to control animals up to week 80. Thereafter, mean body weights were slightly lower ($\leq$ 10%) than those of control animals; the values, however, did not differ significantly from control except for low-dose females at week 98.

3. Food Consumption, Water Consumption, and Compound Intake: Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight data. Compound efficiency (g weight gain/g food consumption) was not calculated by the study authors and could not be calculated by the reviewers because food consumption was determined for individual cages, yet individual animal numbers per cage were not identified. The study authors, however, measured food conversion, expressed as g food consumption/kg body weight/day. Weekly water consumption was measured for weeks 35 to 63 of the study.

Results: Food consumption--Food consumption in male and females receiving the low dose was similar to that of control animals. Food consumption in males and females receiving the high dose was significantly lower than control animals during weeks 1 to 13 of the study; no significant differences were noted thereafter.

Food conversion--Food conversion (expressed as g food consumption/kg body weight/day) was similar among dosed and control animals.

Compound Intake--Compound intake was 0.35 and 1.6 mg/kg body weight for males and 0.36 and 1.6 mg/kg body weight for females.

Water Consumption--Water consumption during weeks 35 to 63 was similar between control and dosed animals. However, a slight trend towards reduced water consumption was noted in males.

4. Ophthalmological examinations were not performed.

5. Blood was collected at weeks 26, 52, and 79 for hematology and clinical analysis from 10 animals/sex/group and on week 104 from 1 to 7 animals/sex/group. The CHECKED (X) parameters were examined.

a. Hematology

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

Reticulocytes, prothrombin time, partial thromboplastin time, and thrombin time were also examined.

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†Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

Results: There were no compound-related effects in the hematology parameters investigated. However, some sporadic differences were noted between dosed and control animals.

b. Clinical Chemistry

Electrolytes		Other	
X	Calcium†	X	Albumin†
X	Chloride†		Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
	Phosphorus†	X	Cholesterol†
X	Potassium†	X	Globulins
X	Sodium†	X	Glucose†
Enzymes		X	Total bilirubin†
X	Alkaline phosphatase (ALP)	X	Total protein†
	Cholinesterase		Triglycerides
	Creatinine phosphokinase†		
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)†		
X	Serum aspartate aminotransferase (also SGOT)†		

Results: There were no compound-related differences between control and dosed animals for the parameters investigated. However, some sporadic differences were observed.

6. Urinalyses: Urine was collected from fasted animals at weeks 26, 52, and 79. The CHECKED (X) parameters were examined.

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 differences between control and dosed animals. However, some sporadic differences were observed.

7. Sacrifice and Pathology: All animals that died and that were sacrificed on schedule (weeks 53, 106, and 116 or 121) were subject to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. The (XX) organs were also weighed.

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†Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerves† (sciatic nerve)
X	Esophagus†	X	Bone marrow†	X	Spinal cord
X	Stomach†	X	Lymph nodes†	XX	Pituitary†
X	Duodenum†	XX	Spleen†	X	Eyes†
X	Jejunum†	XX	Thymus†		Glandular
X	Ileum†		Urogenital	XX	Adrenals†
X	Cecum†	XX	Kidneys†		Lacrimal gland
X	Colon†	X	Urinary bladder†	X	Mammary gland†
X	Rectum†	XX	Testes†		Parathyroids†
XX	Liver†	X	Epididymides	X	Thyroids†
	Gallbladder†	XX	Prostate		Other
X	Pancreas†	X	Seminal vesicle	X	Bone (femur)†
	Respiratory	XX	Ovaries	X	Skeletal muscle†
X	Trachea†	X	Uterus	X	Skin, mammary area
XX	Lung†			X	All gross lesions and masses

Results:

a. Organ weights were determined at weeks 53 (liver, adrenals, brain, heart, kidneys, and gonads) and 106 and at final sacrifice (the six organs mentioned above as well as the thymic remnant, spleen, lung, thyroid, prostate, and pituitary gland). There were no apparent compound-related differences in mean organ weights, organ-to-body weight ratios, and organ-to-brain weight ratios of males and females throughout the study.

However, the mean heart weight and heart-to-brain weight ratio of high-dose females were significantly lower than control values at final sacrifice, and higher mean prostate weights were noted in the high-dose males at week 106. In addition, higher mean prostate weights were noted in high-dose males at final sacrifice, mean ovary weights in low- and high-dose females at final sacrifice, and mean pituitary weights of low- and high-dose females at week 106 when compared to control values. However, these increases may be primarily due to outliers.

b. Gross Pathology: There were no apparent compound-related differences in dosed animals when compared to control animals.

c. Microscopic Pathology:

1) Nonneoplastic: There were no apparent compound-related effects in tissues of dosed animals when compared to control animals.

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†Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

2) Neoplastic: There were no apparent compound-related effects in tissues of dosed animals when compared to controls.

D. STUDY AUTHORS' CONCLUSIONS:

The mean body weights of all dosed males and females in group 3 (30 ppm) were slightly (within 10%) lower than in the respective control group. About the same depression was observed in the previous study at this dose level (30 ppm). The mean body weight of dosed males and females in group 2 (6 ppm) was similar to that of the respective controls.

The mean food consumption of dosed males in group 3 (30 ppm) was slightly (within 10%) lower than in the respective control group, and females in group 3 (30 ppm) also showed a trend to lower food consumption when compared to the female control group. This slight depression is in line with the depression of food consumption at this dose level (30 ppm) during the treatment period of the previous study. The mean food consumption of dosed males and females in group 2 (6 ppm) was similar to that of the respective controls. The mean food conversion ratio of dosed animals was similar to the control ratios, which corresponds to the results of the previous study. Median survival time and mortality distribution of animals in the dosed groups were similar to that of their respective control groups, as could be expected from the results of the previous study.

No clinical symptoms and no signs of local and/or systemic toxicity were observed. Observations for unusual reactions or untoward behavior for both dosed and control animals were similar. Hearing tests performed during the study revealed no treatment-related effects on auditory perception.

Analysis of hematological and biochemical data with significant differences between controls and treated rats revealed that most findings reflect the normal physiological variation of the respective parameters. The findings in the urine were generally unremarkable and comparable to those of the controls.

The analysis of organ weights and organ-to-body and organ-to-brain weight ratios revealed no compound-related differences between the values of dosed and control groups.

Neither gross nor microscopic changes were observed in the organs and tissues examined. Numerous benign and malignant tumours were noted in both control and dosed rats. Frequency and type of neoplasms occurring in these animals were not influenced by dosing with the test material. The apparently observed higher incidence of carcinomas of the mammary gland, which was observed in the 30-ppm female group of the previous study, could not be verified. Similarly, the incidence of foam cells in the lung alveoli and nodular hyperplasia of the Leydig cells in the testes was not increased at the dose levels used in this follow-up study.

The "no observable effect level" for GS 13529, when offered to rats continuously in their feed over a period of 2 years, is 6 ppm, corresponding to a mean daily intake of GS 13529 of 0.35 mg/kg body weight for males and 0.36 mg/kg body weight for females.

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study design was adequate and there were no major problems that compromised the findings. The reporting was good and the summary tables were supported by individual animal data, excluding those for daily observations.

The incidences of mammary gland carcinomas in females were 5, 7, and 4 for the control, low-dose (6 ppm), and high-dose (30 ppm) animals, respectively; similarly, the incidences of mammary gland fibroadenomas were 24, 23, and 28, respectively, for the same three groups. Analyses of these data show no significant increase in the incidence of mammary gland carcinoma noted in females receiving 30 ppm of test material when compared to control animals; thus, the significant increase in these tumors reported in the previous study (No. 785196) is not confirmed by this study.

The following deficiencies in the study were noted. Animals received test diets for 98 weeks and were allowed a recovery period of up to 18 or 23 weeks for males and females, respectively. Thus, certain compound-related effects may not be quite evident after the recovery period. Individual histopathology findings were reported for those tissues with lesions or not examined because of technical problems or cannibalism but not for tissue with no apparent changes, consequently a tissue inventory for individual animals was not presented. Moreover, the number of tissues examined for each group of animals was not reported in the summary table; only the number of animals on study was specified. Nonetheless, visual examination of the data suggests that only a few tissues were not examined.

Based on body weight and food consumption data, the NOEL and LOEL for chronic toxicity are 6 and 30 ppm, respectively, of terbuthylazine in the diet.

CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

CASWELL FILE

005834

EPA: 68-02-4225
DYNAMAC No. 239-A
January 22, 1987

DATA EVALUATION RECORD

TERBUTHYLAZINE (BELCLENE)

Chronic Toxicity/Oncogenicity Study in Rats

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-22-87

CASWELL FILE

EPA: 68-02-4225
DYNAMAC No. 239-A
January 22, 1987

DATA EVALUATION RECORD

TERBUTHYLAZINE (BELCLENE)

Chronic Toxicity/Oncogenicity Study in Rats

REVIEWED BY:

Nicolas P. Hajjar, Ph.D.
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Date: January 20, 1987

William L. McLellan, Ph.D.
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Signature: I Cecil Felkner
Date: 1-22-87

David Van Ormer, Ph.D.
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Signature: D L Van Ormer
Date: Feb 9, 1987

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: Feb 10, 1987

005834

DATA EVALUATION REPORT

Tox Chem No. 125B

MRID NO.: N/A

STUDY TYPE: Chronic toxicity/oncogenicity study in rats.

ACCESSION NUMBER: 261456-261457.

TEST MATERIAL: GS 13529 technical.

SYNONYMS: Terbuthylazine.

STUDY NUMBER(S): 785196.

SPONSOR: CIBA-GEIGY Ltd., Basle, Switzerland.

TESTING FACILITY: Sisseln facility, 4332 Stein, Switzerland.

TITLE OF REPORT: GS 13529--Lifetime carcinogenicity and chronic toxicity study in rats.

AUTHOR(S): Gfeller, W.; Basler, W.; Zak, F.; Hess R.

REPORT ISSUED: June 16, 1983.

CONCLUSIONS:

GS 13529 technical was administered to male and female rats (Tif:RAIf) in the diet at concentrations of 0, 30, 150, or 750 ppm for 104 weeks; thereafter, animals were placed on untreated diets until final sacrifice at week 112 for males and week 122 for females. Thus, the recovery period was up to 9 weeks in males and 18 weeks in females.

Under the conditions of the study, an increased incidence of benign interstitial cell tumors of the testis was observed in males receiving 750 ppm (HDT) and an increased incidence of mammary gland carcinomas was noted in females receiving 30 (LDT) and 750 ppm. There was a compound-related decrease in the mean body weights and food consumption of all dosed males and females. There was a compound-related decrease in mean erythrocyte count, hemoglobin concentration and hematocrit in females receiving 150 and 750 ppm during the first 18 months of the study. Similarly, there was an increase in blood urea nitrogen, a decrease in urine volume, and an increase in specific gravity of the urine in males and females receiving the mid and high doses during the first 18 months. The mean liver, kidney, and heart weights in animals receiving the mid and high doses were lower than control values throughout the study. Nonneoplastic histopathologic changes were observed in males and females receiving the high dose. These included a significant increase in the incidences of inflammatory cell infiltration of the lung, foam cells of the lung alveoli, and nodular hyperplasia of the interstitial Leydig cells of the testis in males and biliary cysts of the liver and nodular hyperplasia of the thyroid gland in females. Based on the available data, a NOEL for chronic toxicity was not achieved, since toxicity was observed at 30 ppm (LDT). The results of a subsequent chronic toxicity study conducted with terbuthylazine at dietary levels of 0, 6, and 30 ppm support this conclusion and are discussed in report No. 791229.

Classification: [redacted] Supplementary

A. MATERIALS:

1. Test Compound: GS 13529; description: white powder; batch No.: 590305; purity: 98% (found to be 96.8% on reanalysis on January 12, 1982); contaminants: not reported.

2. Test Animals: Species: rat, strain: Tif:RAIf (SPF); age: approximately 4 weeks; weight: males--94-96 g, females--91-93 g; source: Animal Production, CIBA-GEIGY, Ltd.

B. STUDY DESIGN:

1. Animal Assignment: Animals were assigned randomly to the following test groups:

Test Group	Dose in Diet (ppm)	Main Study (25-27 Months) Males	Main Study (25-27 Months) Females	Interim Sacrifice (12 and 24 Months) Males	Interim Sacrifice (12 and 24 Months) Females
1 Control	0	80	80	10	10
2 Low (LDT)	30	80	80	10	10
3 Mid (MDT)	150	80	80	10	10
4 High (HDT)	750	80	80	10	10

The animals were acclimated to laboratory conditions for 10 days prior to treatment. The animals were housed five to a cage and placed on diets containing test material for 104 weeks only; thereafter, the animals received control diets. Final sacrifice of males and females was at weeks 112 and 122, respectively.

2. Diet Preparation: Diets were prepared at unspecified periods and stored at an unspecified temperature. Samples of food containing test material were analyzed for stability and concentration at weeks 3, 12, 24, 34, 44, 54, 59, 67, 83, 88, and 101 of the study. Each sample was fed for approximately 1 month. After week 104, all surviving animals were placed on control diets until final sacrifice, males at week 112 and females at week 122.

   Results: Analysis of the test material showed that mean percent concentrations were 86, 87.5 and 88.5% (calculated by the reviewers) of nominal concentrations of the 30-, 150-, and 750-ppm test diets.

3. Animals received food (certified standard diet Nafag) and water ad libitum.

4. Statistics: For each time point and parameter, a univariate statistical analysis was conducted. Each dose group was compared to the control group with respect to dispersion and displacement. In addition, a trend test was applied to all groups. Survival analysis was performed by the generalized Wilcoxon test and the generalized Savage test. The incidence of histopathological lesions was investigated using a method described by R. Peto et al.

5. A quality assurance statement was dated July 13, 1983.

C. METHODS AND RESULTS:

1. Observations: Animals were inspected daily for signs of toxicity and mortality.

   Results: It was reported that there were no compound-related signs of toxicity in dosed rats. Individual animal data were not presented. In males, survival time was significantly ($p < 0.002$, Breslow test) increased in a dose-related manner; it was also slightly but not significantly increased in dosed females (Table 1).

TABLE 1. Survival in Rats Fed Diets Containing GS 13529 for 104 weeks

Test Group	Number of Live Rats at Week					
	0	28	54[a]	80	105[a]	112/122[b]
Males						
Control	80	80	69	61	25	11
30 ppm	80	80	69	61	32	16
150 ppm	80	79	67	64	38	21
750 ppm	80	79	69	67	51	30
Females						
Control	80	80	70	61	41	11
30 ppm	80	80	70	64	48	19
150 ppm	80	79	69	66	48	22
750 ppm	80	76	64	58	41	17

[a]Ten animals from each group were killed at interim sacrifice (weeks 52 and 104 of the study).

[b]Males sacrificed at week 112 and females at week 122 of the study; values were calculated by the reviewers.

Final sacrifice for males and females was at weeks 112 and 122 of the study, respectively.

2. Body Weight: Rats were weighed weekly for 3 months and monthly thereafter.

   Results: The mean body weights of dosed animals were significantly lower in the mid- and high-dose groups by week 1 of the study and in the low-dose group by week 5 (Table 2). Lower body weights were observed in all dosed animals. However, the decreased weights were not statistically significant for the low- and mid-dose groups near the end of the study.

3. Food Consumption, Water Consumption, and Compound Intake: Consumption was determined and mean daily diet consumption was calculated. Compound intake was calculated from the consumption and body weight gain data. Compound efficiency (g weight gain/g food consumption) was not calculated by the reviewers because food consumption was determined for individual cages (containing five animals each); however, individual animals in each cage were not identified by number. The study authors, did however, measure food conversion, which was expressed as food consumption/kg body weight/day. Weekly water consumption was measured for weeks 76 to 104 of the study.

   Results: Food consumption--Food consumption in dosed groups was significantly lower than in the control groups. The decrease was dose dependent, but was less pronounced in dosed females when compared to dosed males (Table 3). Food consumption in dosed animals increased after week 104 of the study, when the animals received control diets.

   Food conversion--Food conversion (expressed as food consumption/kg body weight/day) was generally similar among dosed males and females when compared to their respective controls. However, in the high-dose female group, food conversion was much higher than that of the control group.

   Compound intake--Mean compound intake was 1.24, 6.97, and 41.47 mg/kg/day for males and 1.37, 7.81, and 52.80 mg/kg/day for females in the 30-, 150-, and 750-ppm groups, respectively.

   Water consumption--In males, mean water consumption during weeks 76 to 104 of the study was slightly lower in the 30- and 150-ppm groups and significantly lower in the 750-ppm groups when compared to the control group. In females, there were no compound-related differences, although there was a tendency towards increased water consumption in the 750-ppm group.

4. Ophthalmological examinations were not performed. However, hearing tests were performed during and at the end of the study, and no compound-related effects were observed.

TABLE 2. Mean Body Weights of Rats Fed Diets Containing GS 13529 for 104 Weeks

Test Group	Mean Body Weights (g) at Weeks						
	-1	12	28	54	80	105	110/119[a]
				Males			
Control	95	482	648	807	884	773	691
30 ppm	94	450*	596*	734*	806	717	623
150 ppm	94	393*	503*	606*	668*	626*	643
750 ppm	96	314*	402*	459*	491*	457*	555*
				Females			
Control	92	301	372	458	547	553	525
30 ppm	91	278*	339*	412*	486*	480*	435
150 ppm	93	247*	292*	341*	390*	393*	433
750 ppm	91	208*	238*	262*	276*	271*	357*

[a]Males were last weighed at week 110 and females at week 119 of the study.

*Significantly different from control value ($p < 0.05$).

TABLE 3. Mean Food Consumption of Rats Fed Diets Containing GS 13529 for 104 Weeks

Test Group	Mean Food Consumption (g/animal/week) at Week 1	12	28	54	80	105	110/119[a]
				Males			
Control	144	166	171	175	174	132	149
30 ppm	130*	163	165	159*	163	130	89
150 ppm	121*	150*	149*	150*	152*	144	146
750 ppm	93*	131*	138*	132*	152	145	183
				Females			
Control	106	119	117	114	126	105	107
30 ppm	95*	119	115	108*	114	107	85
150 ppm	87*	117	107	103*	113	111	84
750 ppm	83*	111	106	103*	118*	147	86

[a]Food consumption for males and females was last measured at weeks 110 and 119, respectively.

*Significantly different from control value ($p < 0.05$).

5. Blood was collected at weeks 17, 26, 52, 78, and 104 for hematology and clinical analysis from 10 animals/group/sex. The CHECKED (X) parameters were examined.

a. Hematology

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

Reticulocytes, prothrombin time, partial thromboplastin time, and thrombin time were also examined.

Results: The mean erythrocyte count, hemoglobin concentration, and hematocrit values in dosed females showed a dose-related decrease during weeks 17, 26, 52, and 78 of the study (Table 4). In males, partial thromboplastin time was higher at week 52 of the study in mid- and high-dose animals and at week 26 in high-dose animals when compared to control. Prothrombin time showed a dose-dependent increase at weeks 52 and 104, but not at week 78 of the study. There were no consistent effects in females. Other compound-related effects were not observed.

b. Clinical Chemistry

	Electrolytes		Other
X	Calcium†	X	Albumin†
X	Chloride†	X	Blood creatinine†
	Magnesium†	X	Blood urea nitrogen† (BUN)
X	Phosphorus†	X	Cholesterol†
X	Potassium†	X	Globulins
X	Sodium†	X	Glucose†
	Enzymes	X	Total bilirubin†
X	Alkaline phosphatase (ALP)	X	Total protein†
	Cholinesterase		Triglycerides
X	Creatinine phosphokinase†		
X	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)†		
X	Serum aspartate aminotransferase (also SGOT)† as well as gamma-glutamyl transpeptidase.		

†Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

TABLE 4. Mean Red Blood Corpuscular (RBC) Count, Hemoglobin (HGB) and Hematocrit (HTC) Values of Females Rats Fed Diets Containing GS 13925 for 104 Weeks

Test Group	Parameter		
	RBC (T/L)	HGB (mmol/L)	HCT
		Week 17	
Control	7.7	9.3	0.43
30 ppm	7.6	9.2	0.43
150 ppm	7.4	9.1	0.42
750 ppm	7.1*[a]	8.8*[a]	0.41*[a]
		Week 26	
Control	7.2	8.8	0.40
30 ppm	7.0	8.7	0.38
150 ppm	7.1	8.8	0.39
750 ppm	6.6*[a]	8.2*[a]	0.37*[a]
		Week 52	
Control	7.4	9.1	0.43
30 ppm	7.4	9.1	0.43
150 ppm	7.1*	8.9	0.42
750 ppm	7.0[a]	8.8[a]	0.42
		Week 78	
Control	7.2	8.7	0.42
30 ppm	7.1	8.7	0.42
150 ppm	6.9	8.4*	0.41*
750 ppm	6.7*[a]	8.3*	0.40*
		Week 104	
Control	6.7	8.5	0.41
30 ppm	6.5	8.2	0.40
150 ppm	6.6	8.3	0.41
750 ppm	6.4	8.0	0.40

[a]Significant negative trend ($p < 0.01$).

*Significantly different from control value ($p < 0.05$).

Results: There was a dose-related increase in the concentrations of urea nitrogen in both sexes at weeks 17, 26, 52, and 78, but not at week 104 of the study (Table 5). The increase was more pronounced in dosed females than in dosed males. Other sporadic differences in clinical chemistry parameters were noted in dosed males and females at weeks 17, 26, 52, and 78, but these were not time or dose related. There were no differences noted at week 104 between dosed and control animals.

6. Urinalyses: Urine was collected from fasted animals at weeks 17, 26, 52, 78, and 104. The CHECKED (X) parameters were examined.

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 was a compound-related increase in specific gravity and a decrease in urine volume of the high-dose males and females at weeks 17, 26, 52, and 78. In addition, dosed animals also exhibited a positive trend for specific gravity and a negative trend for urine volumes at weeks 17, 26, 52, and 78. Significantly lower pH values were also noted in high-dose males throughout the study (Table 6).

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 were also weighed.

	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue	X	Aorta†	XX	Brain†
X	Salivary glands†	XX	Heart†	X	Peripheral nerves†
X	Esophagus†	X	Bone marrow†		(Sciatic nerve)
X	Stomach†	X	Lymph nodes†	X	Spinal cord
X	Duodenum†	XX	Spleen†	XX	Pituitary†
X	Jejunum†	XX	Thymus†	X	Eyes†
X	Ileum†		Urogenital		Glandular
X	Cecum†	XX	Kidneys†	XX	Adrenals†
X	Colon†	X	Urinary bladder†		Lacrimal gland
X	Rectum†	XX	Testes†	X	Mammary gland†
XX	Liver†		Epididymides		Parathyroids†
	Gall bladder†	XX	Prostate	XX	Thyroids†
X	Pancreas†		Seminal vesicle		Other
	Respiratory	XX	Ovaries	X	Bone (femur)†
X	Trachea†	X	Uterus	X	Skeletal muscle†
XX	Lung†			X	Skin, mammary area
				X	All gross lesions and masses

†Recommended by Subdivision F (October 1982) Guidelines for chronic studies.

TABLE 5. Mean Blood Urea Nitrogen of Male and Female Rats Fed Diets Containing GS 13925 for 104 Weeks

Test Group	Blood Urea Nitrogen (mmol/L) at Week				
	17	26	52	78	104
Males					
Control	5.0	4.7	4.2	4.2	3.7
30 ppm	5.3	4.8	4.9*	4.7	4.4
150 ppm	5.3	4.9	4.9	6.1	3.5
750 ppm	5.7*	5.7*[a]	6.0*[a]	6.8*[a]	4.6
Females					
Control	5.3	5.2	5.3	4.7	4.5
30 ppm	6.5*	6.3	6.1	5.9*	3.8
150 ppm	6.9*	7.2*	7.4*	6.7*	5.2
750 ppm	7.8*[a]	6.8[a]	8.2*[a]	9.1*[a]	5.5

[a]Significant positive trend ($p < 0.01$).

*Significantly different from control value ($p < 0.05$).

TABLE 6. Mean Urine Volume, Specific Gravity, and pH of Rats Fed Diets Containing GS 13925 for 104 Weeks

Test Group	Volume (mL) M	Volume (mL) F	Specific gravity M	Specific gravity F	pH M	pH F
			Week 17			
Control	8.8	6.6	1.034	1.034	7	6
30 ppm	7.9	7.2	1.035	1.034	6	6
150 ppm	6.7	5.9	1.038	1.035	6*	6
750 ppm	5.0*[a]	4.3*[a]	1.045*[a]	1.040	6*	6
			Week 26			
Control	8.3	6.5	1.037	1.030	7	6
30 ppm	6.9	7.3	1.033	1.030	7	6
150 ppm	7.4*	4.7	1.040	1.038	7	6
750 ppm	6.1*[a]	4.2[a]	1.046[a]	1.046*[a]	6*[a]	6
			Week 52			
Control	9.7	8.8	1.028	1.031	7	6
30 ppm	8.3	8.0	1.028	1.028	7	6
150 ppm	6.0*	7.5	1.040*	1.032	6*	6
750 ppm	5.1*[a]	4.7*[a]	1.043*[a]	1.038*	6*[a]	6
			Week 78			
Control	10.4	9.5	1.025	1.024	7	6
30 ppm	10.7	8.7	1.028	1.026	7	7
150 ppm	7.8	9.9	1.033*	1.026	6	6
750 ppm	8.1	6.5	1.033*[a]	1.029*	6	6
			Week 104			
Control	7.0	9.0	1.029	1.024	7	6
30 ppm	8.6	12.2	1.025	1.023	6*	7
150 ppm	7.0	8.3	1.038	1.027	6*	6
750 ppm	7.5	7.1	1.031	1.034	6*	6

[a]Significant trend ($p < 0.01$).

*Significantly different from control value ($p < 0.05$).

Results:

a. Organ weights were determined at weeks 53 and 106 (brain, heart, liver, kidneys, adrenals, and gonads) and at final sacrifice (the six organs mentioned above, as well as the thymus, spleen, thyroid, lung, and pituitary).

The mean liver, heart, and kidney weights and respective organ-to-brain weight ratios of mid- and high-dose males and females were lower than control animals throughout the study. In addition, the organ-to-body weight ratios were significantly increased for most organs of mid- and high-dose animals throughout the study, as shown in Table 7.

The mean brain and gonad weights of dosed males and females at weeks 53 and 106 were similar to control values. Mean adrenal weights in dosed animals were similar to control values except for, the low-dose males at week 53, which were significantly higher than control value; the mid-dose males at week 106, which were significantly lower (0.091 g); and the high-dose males at week 106, which were significantly higher (0.142 g) than the control value (0.135 g).

At final sacrifice, the mean adrenal weights in high-dose males were significantly lower than control value. In high-dose females, the mean adrenal, thymus, ovaries, and spleen weights were higher than control values, whereas the mean thyroid weights in mid- and high-dose animals were significantly lower. There were no effects noted in mean brain weights.

b. Gross Pathology: There were no apparent compound-related changes in tissues of rats at the 1-year interim sacrifice. At the end of the study, there were no apparent differences in macroscopic findings between controls and dosed animals, except for increased incidences of liver cysts and large spleens in dosed males and females when compared to controls (Table 8).

c. Microscopic Pathology:

1) Nonneoplastic: At the 1-year interim sacrifice, female rats receiving the high dose showed increased incidences of lung congestion and hemorrhage. Cholangiofibrosis of the intrahepatic bile duct and a number of liver and lung lesions were also noted, but the incidences were similar between control and dosed animals. In addition, all dosed females showed nephrocalcinosis. The combined data for the 2-year interim sacrifice, final sacrifice, and animals that died or killed in extremis during the study indicate a significant increase in the incidences of inflammatory cell infiltration

TABLE 7. Selected Mean Organ and Organ-To-Body Weight Ratios of Rats Fed Diets Containing GS 13925 for 104 Weeks

Test Group	Heart		Liver		Kidney	
	M	F	M	F	M	F
			Week 53			
Control	1.69[a]	1.01	22.88	12.17	3.91	2.40
	0.22[b]	0.26	2.97	3.07	0.51	0.61
30 ppm	1.61	0.99	20.22	13.10	3.77	2.36
	0.23	0.26	2.88	3.47	0.54	0.63
150 ppm	1.48*	0.92	18.52*	12.32	3.58	2.19
	0.25*	0.31	3.08	4.04*	0.60*	0.72*
750 ppm	1.23*[c]	0.87*[c]	16.55*[c]	10.68	2.98*[c]	2.00*[c]
	0.28*[c]	0.35*[c]	3.80*[c]	4.22*[c]	0.69*[c]	0.79*[c]
			Week 106			
Control	1.61	1.23	20.08	14.82	4.69	2.94
	0.23	0.25	2.84	2.97	0.68	0.59
30 ppm	1.62	1.22	20.07	14.36	4.56	2.91
	0.24	0.27	2.96	3.16*	0.68	0.66
150 ppm	1.44	1.16	18.94	15.98	4.20	2.93
	0.25	0.29*	3.27*	4.02*	0.73	0.75*
750 ppm	1.20*[c]	0.89*[c]	16.09*[c]	12.69*	3.20*[c]	2.26*[c]
	0.27*[c]	0.33*[c]	3.68*[c]	4.67*[c]	0.74*	0.84*[c]

(Continued)

TABLE 7. Selected Mean Organ and Organ-To-Body Weight Ratios of Rats Fed Diets Containing GS 13925 for 104 Weeks

Test Group	Heart		Liver		Kidney	
	M	F	M	F	M	F
			Final Sacrifice[d]			
Control	1.90 0.32	1.33 0.29	19.59 3.29	16.26 3.59	4.41 0.77	2.94 0.65
30 ppm	1.96 0.35	1.29 0.33	19.07 3.26	15.32* 3.85*	4.46 0.79	3.10 0.80*
150 ppm	1.72 0.30	1.31 0.32	19.30 3.36	16.30* 3.97*	4.08 0.72	2.94 0.72
750 ppm	1.61*[c] 0.30	1.29 0.40*[c]	19.56 3.65*[c]	13.58* 4.06	3.51*[c] 0.67	2.57*[c] 0.79*

(Concluded)

[a]Mean organ weight (g).

[b]Organ-to-body weight ratio.

[c]Significant trend ($p < 0.01$).

[d]Final sacrifice for males and females was at week 112 and at week 122, respectively.

*Significantly different from control value ($p < 0.05$).

TABLE 8. Incidence of Macroscopic Lesions in Rats Fed Diets Containing GS 13529 for 104 Weeks and Observed up to Week 122[a]

Tissue	Males/Dose (ppm)				Females/Dose (ppm)			
	0	30	150	750	0	30	150	750
(No. of animals examined	69	69	67	69	70	70	69	64)
Abdominal cavity								
Mass	-	-	2	1	5	2	2	-
Abdominal wall								
Mass	9	7	2	4	13	15	7	8
Chest wall								
Mass	7	3	7	1	14	14	18	15
Liver								
Cyst	0	0	2	4	0	5	4	10
Mass	1	3	3	0	3	3	2	3
Organs and tissues								
No changes	12	8	19	28	9	12	14	9
Partial cannibalism	9	1	1	0	3	4	1	1
Pituitary gland								
Hemorrhage	12	11	7	8	17	11	12	12
Enlarged	22	29	23	13	31	22	24	22
Spleen								
Enlarged	1	5	4	3	1	4	3	5

[a] Includes 10 animals/sex/group sacrificed at week 106, animals that died or were sacrificed moribund after 52 weeks, and those sacrificed at study termination (males, 112 weeks, and females, 122 weeks).

of the lung, foam cells of the lung alveoli, and nodular hyperplasia of the interstitial Leydig cell of the testis in high-dose males (Table 9). In females, increased incidences of biliary cyst of the liver and nodular hyperplasia of the thyroid gland were noted in the high-dose animals when compared to controls (Table 9). However, the number of tissues examined were not specified.

2) Neoplastic: At the 1-year interim sacrifice, one male from each group receiving 30 and 750 ppm of test material had a benign interstitial cell tumor, and one control male had an adenoma in the thyroid gland. A mammary gland carcinoma was found in a female receiving 750 ppm of test material and a subcutaneous fibroma was found in a female receiving 150 ppm; both animals were killed during the first year of the study. The combined data for the 2-year interim sacrifice, final sacrifice, and animals that died or were killed in extremis during the study indicated increased incidences of mammary gland carcinomas in the low- and high-dose females and an increased incidence of interstitial cell tumors of the testis in the high-dose males when compared to control animals (Table 10). Increased incidences of C-cell adenoma of the thyroid gland were noted in the males receiving the mid and high doses, but the increase was not statistically significant.

D. STUDY AUTHORS' CONCLUSIONS:

The mean body weights of all dosed male and female groups were significantly depressed in a dose-dependent manner. The mean food consumption in dosed males of group 2 (30 ppm) was slightly, but significantly, depressed when compared to the control groups; the effect was greater at higher doses and the response was dose-dependent. A slight depression was also observed in dosed females of groups 3 and 4 (150 and 750 ppm) and during some time periods in females of group 2 (30 ppm). The food consumption in dosed females of group 4 (750 ppm) was subjected to large variation. Survival time of dosed male animals was significantly prolonged in a dose-dependent manner. Survival time of dosed female animals was also slightly, though not significantly, prolonged.

No clinical symptoms and no signs of local and/or systemic toxicity were observed. Observations for unusual reactions or untoward behavior for both treated and control animals were similar. Hearing tests performed during and at the end of the dosing period revealed no compound-related effects on auditory perception.

A slight decrease in the number of erythrocytes was observed in females of group 4 (750 ppm) at weeks 17, 28, and 78. A slight decrease of hemoglobin concentration and hematocrit was seen in females of group 4 (750 ppm) at weeks 17, 26, and 78 and in females of group 3 (150 ppm) at week 78. The prothrombin time was prolonged in males of group 4 (750 ppm) at week 104. In spite of these hematological findings, no consistent trend was perceived, which would clearly indicate an influence of the treatment on hematopoiesis.

TABLE 9. Incidence of Selected Nonneoplastic Histopathologic Findings in Rats Fed Diets Containing GS 13529 for 104 Weeks and Observed up to Week 122

Tissue	Males/Dose (ppm)				Females/Dose (ppm)			
	0	30	150	750	0	30	150	750
(No. of animals examined[a]	69	69	67	69	70	70	69	64)
Spleen								
Hemosiderosis	27	29	26	23	31	25	28	38
Hematopoiesis	10	8	7	4	16	13	18	21
Lung								
Congestion	19	26	18	10	14	12	7	9
Edema	8	12	7	5	9	5	4	2
Inflammatory cell infiltration	13	19	7	23*	7	11	11	5
Lung alveolus								
Foam cell	24	24	23	46*	8	12	14	35*
Liver								
Biliary cyst	1	-	3	2	4	9	10	14*
Congestion	15	12	8	10	12	8	6	5
Inflammatory cell infiltration	20	17	11	9	10	12	7	3
Fatty change	20	14	5	3	26	21	11	4
Necrosis	22	18	12	12	14	13	12	5
Interhepatic bile duct								
Cholangiofibrosis	10	13	21	7	23	13	10	20
Kidney								
Congestion	4	14	5	6	4	5	3	2
Chronic progressive neophropathy	50	43	42	24	41	42	40	34
Nephrocalcinosis	-	-	-	-	64	66	62	64
Urinary bladder								
Chronic inflammation	5	7	5	3	2	-	1	1
Inflammatory cell infiltration	10	7	1	2	-	-	1	-

(Continued)

TABLE 9. Incidence of Selected Nonneoplastic Histopathologic Findings in Rats Fed Diets Containing GS 13529 for 104 Weeks and Observed up to Week 122 (Continued)

Tissue	Males/Dose (ppm)				Females/Dose (ppm)			
	0	30	150	750	0	30	150	750
Testis interstitial Leydig cell nodular hyperplasia	6	3	5	21*				
Adrenal gland Sinusoid cystic dilatation	5	3	-	2	33	39	43	31
Adrenal cortex Nodular hyperplasia	18	20	13	9	11	5	6	4
Thyroid gland Nodular hyperplasia	1	2	1	1	-	2	1	4*

(Concluded)

[a]The numbers of tissues examined were not specified.

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

TABLE 10. Incidence of Selected Neoplastic Histopathologic Findings in Rats Fed Diets Containing GS 13529 for 104 Weeks and Observed up to Week 122

Tissue	Males/Dose (ppm)				Females/Dose (ppm)			
	0	30	150	750	0	30	150	750
(No. of animals examined[a]	69	69	67	69	70	70	69	64)
Mammary gland								
Carcinoma	2	1	2	-	4	9*	3	13*
Fibroadenoma	2	-	1	-	16	17	9	8
Lymph node								
Malignant lymphoma, systemic infiltration	-	-	4	1	2	-	1	2
Testis								
Benign interstitial cell tumor	3	3	2	9*				
Ovary								
Benign cell tumor					7	8	6	5
Pituitary gland								
Adenoma	27	34	26	20	28	28	29	23
Thyroid gland								
C-cell adenoma	1	1	5	4	2	4	3	3
C-cell carcinoma	3	1	1	-	1	1	-	-

[a]The number of tissues examined was not specified.

*Significantly different from control value ($p < 0.05$).

A slight increase in the urea nitrogen concentrations were seen in males of group 4 (750 ppm) at week 78, in females of group 4 (750 ppm) at weeks 17, 52, and 78, and in females of group 3 (150 ppm) at weeks 17, 26, and 52. A slight increase in the gamma-globulin fraction was observed in females of group 4 (750 ppm) at weeks 52 and 78. However, these biochemical findings do not indicate a compound-related toxic effect on liver functions.

Urine volume decreased in males of group 4 (750 ppm) at weeks 17 and 52, in males of group 3 (150 ppm) at week 52, in females of group 4 (750 ppm) at weeks 17, 26 and 52, and in the females of group 3 (150 ppm) at week 26.

The specific gravity of urine increased in males of group 4 (750 ppm) at weeks 17, 52, and 78, in males of group 3 (150 ppm) at weeks 52 and 78, and in females of group 4 (750 ppm) at weeks 17, 26, and 52. Individual rats including those of the control group revealed proteinuria and erythrocytes and leukocyte in the sediment.

The authors also concluded that all observed variations in absolute and relative organ weights were associated with the concurrent decrease of body weight development, except a minimal decrease of the weight of the adrenals at final sacrifice in males of group 5 (750 ppm). In females of group 4 (750 ppm) and of group 2 (30 ppm), a higher incidence of carcinomas of the mammary gland was noted. However, in group 4 (750 ppm) this finding was associated with a lower incidence of fibroadenomas of the mammary gland. Consequently, the total number of females bearing a tumor of the mammary gland was not systematically affected by the exposure to the test compound. Foam cells in lung alveoli occurred in significantly increased incidences in both male and female rats of group 4 (750 ppm). The incidence of nodular hyperplasia of the Leydig cells in the testes was found to be increased in males of group 4 (750 ppm).

It can be inferred from the observations made during the course of the above study that a "no observable effect level" for GS 13529, when offered to rats continuously in their diet over a period of 2 years, is below 30 ppm. Therefore, a subsequent study with lower dose levels of 0, 6, and 30 ppm was started on October 29, 1979 (project No. 79122).

E. REVIEWERS' DISCUSSION AND INTERPRETATION OF RESULTS:

The study was of adequate design to test the oncogenicity and chronic toxicity of terbuthylazine and there were no major problems that would compromise the findings. The reporting was good and summary tables were supported by individual animal data, except for daily observations. We agree with most of the conclusions and interpretations of the report authors. However, hematology findings in females and blood urea nitrogen concentrations and urinalysis findings suggest compound-related effects during the first 18 months of the study. These effects, namely anemia and impaired formation or excretion of

urine, are supported by body weight decreases and histologic findings in the kidneys of dosed animals. The significantly increased organ-to-body weight ratios indicate the possibility of organ-specific effects in addition to general body weight depression. Based on these findings as well as the significantly lower mean body weights of all dosed male and female rats throughout the 104-week dosing period, a MTD was achieved in this study.

The significance of food conversion expressed as food consumption/kg body weight/day is not clear. Food efficiency is a better indicator. Since individual animal data were not available, a rough estimate for weeks 1-12 can be made using mean weight gain/mean food consumption. The data indicate decreased food efficiency in mid- and high-dose males and females (0.189, 0.189, 0.175, and 0.145 g weight/g food consumption in males and 0.138, 0.135, 0.116, and 0.089 g weight gain/g food consumption in females receiving 0, 30, 150, or 750 ppm of test the material, respectively.)

In addition, several deficiencies were noted. (1) The animals receiving the test material were allowed a recovery period beyond week 104 of the study. Thus, the recovery period was for up to 8 and 18 weeks in males and females, respectively. Although this recovery period may have modified histopathologic findings its effect on tumorigenesis would be minimal, as indicated by the increased incidences of mammary tumors in females and interstitial cell tumors of the testis in males which are age related. (2) Individual histopathology findings were reported for those tissues with lesions or not examined because of technical problems or cannibalism; thus, a tissue inventory for individual animals was not presented. Moreover the number of tissues examined for each group of animals was not reported in the summary table; only the number of animals was specified. Nonetheless, visual examination of the data suggests that only a few tissues were not examined.

In agreement with the authors' conclusions, a NOEL was not achieved in this study. The NOEL for this study is below 30 ppm (LDT) of test material in the diet, based on lower body weights of animals receiving the low dose.