EPA Reviewer: Edwin Budd, M.S.
Registration Action Branch 2 (7509C)

Edwin Budd, Date 2/14/01

014613

This Data Evaluation Record (DER) includes the original DER prepared for this study by Oak Ridge National Laboratory (Attachment #1) and an excerpt from the Cancer Assessment Document prepared by the Cancer Assessment Review Committee (HED) following its evaluation of the carcinogenic potential of fluazinam on January 3, 2001 (Attachment #2). The updated Executive Summary presented below contains pertinent data and information from both attachments.

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity feeding study – Rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D258235
P.C. CODE: 129098

SUBMISSION CODE: S561478
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): B-1216 (Fluazinam)(95.3% a.i.)

SYNONYMS: 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine, IKF-1216, PP192

CITATION: Mayfield, R., S. Burton, D. Crook, et al. 1988. Fluazinam technical (B1216): potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks. Huntingdon Research Centre, Ltd., Cambridgeshire, PE18 6ES, England. Report No. ISK 8/87263, August 25, 1988. MRID 42248620. Unpublished.

Mayfield, R., C. Gopinath, and S. Begg. 1999. Addendum to report No. ISK 8/87263. B-1216: potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks. Huntingdon Life Sciences, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Report No. ISK 8/87263 Addendum, March 3, 1999. MRID 44807223. Unpublished.

Lewis, D.L. 2000. Supplement to report no. ISK 8/87263 (MRID#42248620) B-1216: potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks (historical control data). Huntingdon Life Sciences, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Document No. ISK 8/87263 Supplement, May 31, 2000. MRID 45150201. Unpublished.

SPONSOR: Ishihara Sangyo Kaisha, Ltd, 10-30, Fujimi 2-chome, Chiyoda-ku, Tokyo 102, Japan

SUBMITTED BY: ISK Biosciences Corporation, 5970 Heisley Road, Suite 200, Mentor, Ohio 44060

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 42248620, 44807223, 45150201), fluazinam technical (95.3% a.i., lot number 8412-20) was administered to groups of 60 male and 60 female Sprague-Dawley rats at dietary concentrations of 0, 1, 10, 100, or 1000 ppm (0, 0.04, 0.38, 3.8, or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.9, or 53 mg/kg/day for females) for up to 104 weeks. Groups of 10 rats of each sex per dose group were sacrificed at 52 weeks for interim evaluations.

No treatment-related effects were observed in rats receiving the 1 ppm or 10 ppm diets. No treatment-related effect on mortality was observed in rats receiving any dose of the test material. The only clinical signs observed were straw-discoloration of the fur in all rats receiving the 1000 ppm diet and an increased incidence of alopecia in females receiving the 1000 ppm diet.

Males receiving the 1000 ppm diet weighed 6–16% ($p<0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed ≤8% less food than controls at each weekly interval. Females receiving the 1000 ppm diet weighed 7–24% ($p<0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed ≤18% less food than controls at each weekly interval. The food utilization factor or the food efficiency suggested that reduced body weight gain was due in part to toxicity of the test material. No treatment-related effects were observed on body weights, body weight gain, food consumption, or food utilization/efficiency in male or female rats receiving the 1-, 10- or 100-ppm diets. No treatment-related effects were observed on the eyes at any dose at any time during the study. Clinical pathology evaluations showed only mild anemia and elevated cholesterol in both sexes receiving the 1000 ppm dose.

Treatment-related microscopic findings showed that the test material was toxic to the following organs: liver, pancreas, lungs, and possibly thyroid gland in both sexes, kidneys and testes in males, and lymph nodes in females, but the primary target appeared to be the liver. Liver toxicity was manifested by increased organ weight and increased incidences of gross and microscopic lesions. Absolute liver weights were increased by at least 7-27% and relative weights by 17-63% in both sexes at 1000 ppm. Gross lesions in the liver consisted of pale, swollen or accentuated markings on the livers at 1000 ppm in males and enlarged, pitted, or mottled livers at 1000 ppm in females. Treatment-related microscopic liver lesions at 100 ppm consisted of eosinophilic hepatocytes in 22% of females (8% in controls), centrilobular hepatocyte rarefaction and vacuolation in 8% of each sex (0% for controls), centrilobular sinusoidal dilatation in 10% of males and 18% of females (0% for male and 2% for female control), and pericholangitis in 18% of males and 14% of females (4% for male and 2% for female controls). Additional treatment-related liver lesions at 1000 ppm in main study group consisted of centrilobular hepatocyte vacuolation in males, centrilobular hepatocyte necrosis in females, and centrilobular fat and bile duct hyperplasia in both sexes. Centrilobular hepatocyte

vacuolation and centrilobular fat was also seen in 1000 ppm group male and female rats at interim sacrifice.

The incidences of exocrine atrophy of the pancreas in both sexes and acinal epithelial vacuolation or fat accumulation in females were increased at 1000; the incidence of exocrine atrophy was also increased at 100 ppm in females compared with that of control rats. The incidence of exocrine degranulation was increased in 1000 ppm group females rats at interim sacrifice but not in the main study. An increased incidence of thyroid follicular hyperplasia was observed in males at 1000 ppm (8% vs 2% for controls) and in females at 1000 ppm (10% vs 2% in controls). This finding may possibly be related to treatment with the test material. In male rats, the incidence of cortical tubular basophilia in the kidney was increased at 1000 ppm compared with that of the controls. Other treatment-related lesions included pneumonitis, alveolar adenomatosis, and alveolar epithelialization in 1000 ppm group males, alveolar epithelialization and alveolar macrophage aggregates in 1000 ppm group females, testicular atrophy in 100 ppm and 1000 ppm group males, and spermatocele granuloma also in 1000 ppm males. The incidence of sinus histiocytosis in the lymph nodes was increased in1000ppm group females. Histopathologic assessment of the brain and spinal cord of rats in the control and 1000 ppm dose groups showed no treatment-related effect on vacuolation of white matter.

The lowest-observed-adverse-effect level (LOAEL) for fluazinam was 100 ppm (3.8 mg/kg/day for males and 4.9 mg/kg/day for females) based on liver toxicity in both sexes, testicular atrophy in males and pancreatic exocrine atrophy in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm (0.38 mg/kg/day for males and 0.47 mg/kg/day for females).

In this study, there were statistically significant positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/adenocarcinomas for the male rats. There was also a statistically significant increase by pair-wise comparison of the male high dose group (1000 ppm) with the controls for combined follicular cell adenomas/adenocarcinomas (23% vs 8% in controls). In addition to an Exact Trend Test and a Fisher's Exact Test, a Peto's Prevalence Test was also conducted (which excluded animals that died or were sacrificed before observation of the first tumor at week 68). For follicular cell adenocarcinomas in males, results of the Peto's Prevalence Test showed a statistically significant positive trend and a borderline statistically significant ($p = 0.056$) increase by pair-wise comparison of the 1000 ppm male group with the controls (7% vs 0% in controls), indicating the increased incidence of thyroid tumors had a malignant component to it. For combined follicular cell adenomas/adenocarcinomas, Peto's Prevalence Test also showed a statistically significant increase by pair-wise comparison of the high dose male group with the controls (26% vs 9% in controls). The incidences of thyroid gland adenomas at 100 ppm and 1000 ppm (15% and 17%, respectively) and adenocarcinomas at 1000 ppm (6%) were slightly outside their respective ranges in the historical control data (range: adenomas, 0%-13%; adenocarcinomas, 0%-5%). Animals in the lower dose groups were not microscopically examined for thyroid lesions unless abnormalities were observed in that organ at gross necropsy. Therefore, percentage incidences of thyroid tumors in these lower dose groups may have been somewhat misleading (too high). The highest dose level tested in this study was considered to be adequate and not excessive because there were decreased body

weight gains (up to 15% and 35% in males and females, respectively), decreased food consumption, decreased food efficiency, increased thyroid weights at 52 weeks, enlarged thyroids and a slightly increased incidence of thyroid gland follicular cell hyperplasia at 104 weeks in males. The survival of the animals was not decreased by treatment with the test material. There was no treatment-related increase in the thyroid tumor incidence in the female rats in this study. In addition, slightly increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats, but these incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). Further, this type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in males and females and of mammary gland fibroadenomas in females were also observed in all groups, including controls. These neoplasms are not considered to be treatment-related. A toxicologically significant increase in tumors was not observed in any other tissues in the treated male or female rats in this study.

This combined chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies guideline requirements for a chronic toxicity/carcinogenicity study [OPPTS 870.4300 (§83-5)] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A signed and dated Flagging statement was included in the addendum.

RAB3001:42248620.der

Attachment #1

Original DER prepared for this study by Oak Ridge National Laboratory

DATA EVALUATION REPORT

FLUAZINAM TECHNICAL
MRID 42248620, 44807223

STUDY TYPE: CHRONIC TOXICITY/ONCOGENICITY ORAL STUDY
[OPPTS 870.4300 (§83-5)]

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 99-51Q

Primary Reviewer: K.A. Davidson, Ph.D., D.A.B.T.	Signature: Date:	DEC 2 3 1999
Secondary Reviewers: Carol S. Forsyth, Ph.D., D.A.B.T.	Signature: Date:	DEC 2 3 1999
Robert H. Ross, Group Leader, M.S.	Signature: Date:	DEC 2 3 1999
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	DEC 2 3 1999

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed by Lockheed Martin Energy Research Corporation for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464

EPA Reviewer: E. Budd, M.S. Edwin Budd, Date 12/11/00
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: M. Copley, D.V.M., D.A.B.T., Date 6/18/01
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/oncogenicity feeding – Rat [OPPTS 870.4300 (§83-5)]

DP BARCODE: D258235
P.C. CODE: 129098

SUBMISSION CODE: S561478
TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): B-1216 (Fluazinam)(95.3% a.i.)

SYNONYMS: 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine, IKF-1216, PP192

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SPONSOR: Ishihara Sangyo Kaisha, Ltd, 10-30, Fujimi 2-chome, Chiyoda-ku, Tokyo 102, Japan

SUBMITTED BY: ISK Biosciences Corporation, 5970 Heisley Road, Suite 200, Mentor, Ohio 44060

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EXECUTIVE SUMMARY: In a combined chronic toxicity/oncogenicity study (MRID 42248620, 44807223, 451450201), B-1216 (Fluazinam technical, 95.3% a.i., lot number 8412-20) was administered to groups of 60 male and 60 female Sprague-Dawley rats at dietary concentrations of 0, 1, 10, 100, or 1000 ppm (0, 0.04, 0.38, 3.82, or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.87, or 53 mg/kg/day for females) for up to 104 weeks. Groups of 10 rats of each sex per dose group were sacrificed at 52 weeks for interim evaluations.

No treatment-related effects were observed in rats receiving the 1- or 10-ppm diets. No treatment-related effect on mortality was observed in rats receiving any dose of the test material. The only clinical signs observed were straw-discoloration of the fur in all rats receiving the 1000-ppm diet and an increased incidence of alopecia in females receiving the 1000-ppm diet.

Males receiving the 1000-ppm diet weighed 6–16% ($p<0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed ≤8% less food than controls at each weekly interval. Females receiving the 1000-ppm diet weighed 7–24% ($p<0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed ≤18% less food than controls at each weekly interval. The food utilization factor or the food efficiency suggested that reduced body weight gain was due in part to toxicity of the test material. No treatment-related effects were observed on body weights, body weight gain, or food consumption, or food utilization/efficiency in male or female rats receiving the 1-, 10- or 100-ppm diets. No treatment-related effects were observed on the eyes at any dose at any time during the study. Clinical pathology evaluations showed only mild anemia and elevated cholesterol in both sexes receiving the 1000-ppm dose.

Treatment related microscopic findings showed that the test material was toxic to the following organs: liver, pancreas, lungs, and possibly thyroid gland in both sexes, kidneys and testes in males, and lymph nodes in females, but the primary target appeared to be the liver. Liver toxicity was manifested by increased organ weight and increased incidences of gross and microscopic lesions. Absolute liver weights were increased by at least 7-27% and relative weights by 17-63% in both sexes at 1000 ppm. Gross lesions in the liver consisted of pale, swollen or accentuated markings on the livers at 1000 ppm in males and enlarged, pitted, or mottled livers at 1000 ppm in females. Treatment-related microscopic liver lesions at 100 ppm consisted of eosinophilic hepatocytes in 22% of females (8% in controls), centrilobular hepatocyte rarefaction and vacuolation in 8% of each sex (0% for controls), centrilobular sinusoidal dilatation in 10% of males and 18% of females (0% for male and 2% for female control), and pericholangitis in 18% of males and 14% of females (4% for male and 2% for female controls). Additional treatment-related liver lesions at 1000 ppm in main study group consisted of centrilobular hepatocyte vacuolation in males, centrilobular hepatocyte necrosis in females, and centrilobular fat and bile duct hyperplasia in both sexes. Centrilobular hepatocyte vacuolation and centrilobular fat was also seen in 1000–ppm group male and female rats at interim sacrifice.

The incidences of exocrine atrophy of the pancreas in both sexes and acinal epithelial vacuolation or fat accumulation in females were increased at 1000; the incidence of exocrine atrophy was also increased at 100 ppm in females compared with that of control rats. The incidence of

exocrine degranulation was increased in 1000-ppm group females rats at interim sacrifice but not in the main study. An increased incidence of thyroid follicular hyperplasia was observed in males at 1000 ppm (8% vs 2% for controls) and in females at 1000 ppm (10% vs 2% in controls). This finding may possibly be related to treatment with the test material. In male rats, the incidence of cortical tubular basophilia in the kidney was increased at 1000 ppm compared with that of the controls. Other treatment-related lesions included pneumonitis, alveolar adenomatosis, and alveolar epithelialization in 1000-ppm group males, alveolar epithelialization and alveolar macrophage aggregates in 1000-ppm group females, testicular atrophy in 100- and 1000-ppm group males, and spermatocele granuloma also in 1000-ppm males. The incidence of sinus histiocytosis in the lymph nodes was increased in 1000-ppm group females. Histopathologic assessment of the brain and spinal cord of rats in the control and 1000-ppm dose groups showed no treatment-related effect on vacuolation of white matter.

The lowest-observed-adverse-effect level (LOAEL) for B-1216 was 100 ppm (3.8 mg/kg/day for males and 4.9 mg/kg/day for females) based on liver toxicity in both sexes, testicular atrophy in males and pancreatic exocrine atrophy in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm (0.38 mg/kg/day for males and 0.47 mg/kg/day for females).

A slightly increased incidence of thyroid gland follicular cell adenomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 100 ppm and 1000 ppm. The incidence was 8%, 6%, 10%, 14% and 14% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 13% and the mean was 6.5%. Also, a slightly increased incidence of thyroid gland follicular cell adenocarcinomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 1000 ppm. The incidence was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 5% and the mean was 1.4%. In addition, slightly increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats, but these incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). Further, this type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in males and females and of mammary gland fibroadenomas in females were also observed in all groups, including controls. These neoplasms are not considered to be treatment-related. A noteworthy increase of tumors was not observed in any other tissues in the treated male or female rats in this study.

This combined chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies guideline requirements for a chronic toxicity/carcinogenicity study [OPPTS 870.4300 (§83-5)] in rats.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. A signed and dated Flagging statement was included in the addendum.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: B1216 (fluazinam technical)

   Description: pale yellow powder
   Lot/Batch #: 8412-20
   Purity: 95.3 % a.i.
   Stability of compound: stable for the duration of the study
   CAS #: 79622-59-6
   Storage: in dark at room temperature

2. Vehicle and/or positive control None

3. Test animals

   Species: rat
   Strain: Sprague-Dawley
   Age and weight at study initiation: ~47 days old; males 194-288 gm ; females 143-206 gm
   Source: Charles River Breeding Laboratories, Portage, MI, USA
   Housing: 5 per cage, Bowman suspended cages made of galvanized iron with a grid floor (26 × 51 × 21 cm)
   Diet: Labsure Laboratory Animal Diet No. 2, *ad libitum*
   Water: Tap water, *ad libitum*
   Environmental conditions:
   Temperature: 21°C
   Humidity: 50%
   Air changes: not reported
   Photoperiod: 12 hour light/12 hour dark
   Acclimation period: 19 days

B. STUDY DESIGN

1. In life dates

   Start: February 5, 1985; end: February 19, 1987

2. Animal assignment

Animals were assigned randomly to the test groups in Table 1 based on body weight stratification after equal elimination of animals from both extremes.

TABLE 1: Study design

Test Group	Conc. in Diet[a] (ppm)	Dose to animal (mg/kg/day)		Main Study 24 months		Interim Sac. 12 months	
		male	female	male	female	male	female
1, Control	0	0	0	50	50	10	10
2, Low	1	0.04	0.05	50	50	10	10
3	10	0.38	0.47	50	50	10	10
4	100	3.82	4.87	50	50	10	10
5, High	1,000	40	53	50	50	10	10

Data taken from pages 18 and 32 MRID 42248620.
[a]Based on 95.3% a.i.

The test material was administered in the feed to satellite group animals for 52 weeks and to main study animals for 104 weeks.

3. Dose selection rationale

No dose selection rationale was described. The dosage levels were specified by the sponsor.

4. Diet preparation and analysis

Diets were prepared each week by grinding the test material with Labsure Laboratory Animal Diet No. 2 and mixing in an inflated polyethylene bag for at least 3 minutes to prepare a premix. The premix was diluted with appropriate amounts of feed and mixed in a double cone blender for at least 7 minutes. Diets were stored at 4°C until used. Before study initiation, homogeneity was tested on samples taken while discharging the low- and high-dosage level preparations from the blender. Samples were taken from the top, middle and bottom levels. Stability was tested on low- and high-dosage samples stored for 21 or 35 days at room temperature or 14, 21 or 28 days at 4°C. During the study, samples of treated food were taken at 2 week intervals for analysis of dietary concentration of test material.

Results –

Homogeneity analysis: 1 ppm: The concentrations of test material in samples taken from the top, middle, and bottom of the blender ranged from 1.12 to 1.30 ppm (mean of duplicate samples) for one preparation and 1.02 to 1.11 ppm for another 1 ppm preparation; the relative standard deviations were 5.66 and 2.99. **1000 ppm**: The concentrations ranged from 978 to 1010 ppm, with a standard deviation of 1.18.

Stability analysis: **1 ppm**: An 8% loss was noted after 21 days and a 17% loss after 35 days at room temperature. No loss was noted after storage for 28 days at 4°C. **1000 ppm**: a 5 and 9% loss of test material was noted after storage for 21 and 35 days, respectively, at room temperature. No loss was noted after storage for up to 28 days at 4°C.

Concentration analysis: The concentration of test material in a large number of the 1-ppm samples showed variances 11 to 43% greater than the target concentration; one was 53% less than the target. All 10-ppm samples were within ± 10% of the target except for two, which were +11 and +17% of the target. The 100- and 1000-ppm samples were within ±11% of the target.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable except for the 1-ppm preparations.

5. Statistics

All parameters: heterogeneity of variance between treatments was analyzed by Levene's test, and comparison of treatment groups with the control was conducted with William's test.

Body weights: the study authors used a very complicated statistical test in which the rats of each sex were assigned to 60 blocks based on body weights. A description of the block system can be found on page 17 and the statistical test on pages 27 and 28 (MRID 42248620). Only mean body weight for weeks 12-13, 25-27, 51-53, 77-79, and 101-104 were analyzed statistically.

Food consumption: statistical analysis was conducted for consumption weeks 1-13, 14-26, 27-52, 53-78, and 79-104.

Hematology and urinalysis parameters were subjected to logarithmic transformation in cases of significant heterogeneity of variance. Fisher's exact test in combination with Mantel's test for trend in proportions was used to analyze eosinophil and monocyte data.

C. METHODS

1. Observations

Animals were inspected daily on weekdays for signs of toxicity for the first 4 weeks and once weekly thereafter; the animals were palpated at the time of inspection. The animals were checked twice daily on week days and weekends for mortality.

2 Body weight

Animals were weighed at the time of allocation to test groups, on the day treatment was initiated, and once a week thereafter.

3. Food consumption and compound intake

Food consumption for each cage was determined on a weekly basis, and mean daily diet consumption was calculated as g food/animal/week. Food conversion ratios (g food/g weight gained) were calculated for the first 26 weeks of the study. Compound intake (mg/kg/day) values were calculated from food consumption and body weight data.

4. Ophthalmoscopic examination

Eyes of 10 male and 10 female rats in the control and 1000-ppm dietary group were examined before treatment was initiated, and during weeks 26, 52, 78, and 101.

5. Blood was collected after light anesthesia from the orbital sinus of 10 male and 10 female rats per group during weeks 13, 26, 52, 78, and 102 for hematology and clinical chemistry analysis. Food was removed overnight before bleeding. The CHECKED (X) parameters were examined. Blood smears from animals selected for clinical pathology and from all animals killed *in extremis* or at scheduled times were prepared for examination of cell morphology.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*		
X	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for chronic toxicity/oncogenicity based on Subdivision F Guidelines

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b. Clinical chemistry

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Total Cholesterol
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
			Total bilirubin
		X	Total serum protein*
	ENZYMES		Triglycerides
X	Alkaline phosphatase (ALK)		
	Cholinesterase (ChE)		
	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase* (also SGPT)		
X	Serum aspartate amino-transferase* (also SGOT)		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

6. Urinalysis

Overnight samples of urine were collected from 10 rats per sex per dose at weeks 12, 25, 51, 77, and 101. Food and water were withdrawn overnight. The same animals were used as selected for blood sampling. The CHECKED (X) parameters were examined.

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

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines

7. Sacrifice and pathology

All animals that died, those sacrificed *in extremis*, or those sacrificed on schedule by carbon dioxide asphyxiation at 52 weeks or study termination were subjected to a detailed gross pathological examination. The CHECKED (X) tissues were collected and preserved for microscopic examination. All collected tissues, gross lesions, and masses from control and 1000-ppm group animals were examined microscopically.

In addition, the following tissues for all low and intermediated group animals were examined microscopically: lung, liver, kidney, and any tissue having possibly treatment-related changes (pancreas, lymph nodes, ovaries, and testes), and all tissues from animals dying or killed before scheduled termination. Frozen sections of liver from all control and 1000-ppm group and rats dying early or killed before scheduled termination in the remaining groups and pancreas from all rats surviving to scheduled termination in all groups were stained with Oil Red O for detection of fat. In an addendum (MRID 44807223), additional sections of brain and spinal cord were taken from all animals in the control and 1000-ppm group for assessment of vacuolation of white matter. The (XX) organs were weighed. The thyroid was weighed after fixation.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*	X	Eyes (optic n.)*
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*		UROGENITAL	XX	Adrenal gland*
X	Colon*	XX	Kidneys*+	X	Lacrimal gland
X	Rectum*	X	Urinary bladder*	X	Mammary gland*
XX	Liver*+	XX	Testes*+	X	Parathyroids*
X	Pancreas*	X	Epididymides	XX	Thyroids*
		X	Prostate	X	Harderian gland
	RESPIRATORY	X	Seminal vesicle	X	Zymbal's gland
X	Trachea*	XX	Ovaries*		
X	Lung*	X	Uterus*		OTHER
X	Nose	X	Cervix	X	Bone*
X	Pharynx	X	Vagina	X	Skeletal muscle*
X	Larynx			X	Skin*
				X	All gross lesions and masses*

*Required for chronic toxicity/oncogenicity studies based on Subdivision F Guidelines.
+Organ weight required in chronic toxicity/oncogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity

Clinical signs were not summarized in tabular form. According to the study authors, clinical signs occurring with increased frequency in rats treated with the test material included a slight to moderate straw discoloration of the fur in both sexes fed the 1000-ppm diet (from week 19 to study termination) and alopecia in 1000-ppm group females surviving to study termination.

2. Mortality

No treatment-related differences in survival were observed in either male or female rats receiving the test material. Survival at 78 weeks was 82, 80, 82, 90, and 72% for male rats in the control, 1, 10, 100, and 1000 ppm groups, respectively, and 78, 90, 90, 90, and 88%, respectively, for female rats. At study termination survival was 28, 36, 32, 44, and 44%, respectively, for male rats and 36, 50, 48, 42, and 60%, respectively, for females. These data showed that terminal survival rates for both sexes receiving the highest doses of test material exceeded that of the controls.

B. BODY WEIGHT

Selected mean body weights and body weight gain data are summarized in Table 2. Male and female rats receiving the 1000-ppm dietary concentration weighed less than controls throughout the study. The males weighed 6–16% less than the controls from week 8 to study termination, and the females weighed 7–24% less than the controls from week 2 to study termination. Statistical analysis was performed on data for the intervals of weeks 12–14, 25–27, 51–53, 77–79, and 102–104; body weights were significantly ($p<0.01$) less than that of controls for 1000-ppm group male and female rats except for the 102-104 interval for male rats. At study termination, 1000-ppm group male rats weighed 10% less than controls and 1000-ppm group females weighed 24% less than controls. Male and female rats in the 1-, 10-, and 100-ppm group had body weights similar to that of the controls throughout the study. Male and female rats in the 1000-ppm group gained less weight than controls; this was first noted during the initial 13 weeks of the study when the males gained 14% less weight than controls and the females gained 29% less. The 1000-ppm group males and females, respectively, gained 17% and 33% less weight than the controls during the first year, 13% more and 37% less during the second year, and 15% and 35% less over the entire study duration

TABLE 2. Selected mean body weights of male and female rats receiving B-1216 for up to 104 weeks

Week of study	Dietary concentration (ppm)									
	Males					Females				
	0	1	10	100	1000	0	1	10	100	1000
Body weights (g)										
0	242	243	242	243	242	170	169	170	169	169
1	294	295	294	294	289 (98)[a]	190	190	190	189	183 (96)
8	475	478	474	469	447 (94)	277	273	272	267	244 (88)
13	533	533	531	523	495 (93)	298	296	293	289	260 (87)
12-14[b]	533	534	534	524	497** (93)	299	295	293	291	261** (87)
26	628	627	626	609	571 (91)	346	341	340	336	291 (84)
25-27[b]	631	629	629	611	573** (91)	346	342	341	337	292** (84)
52	745	748	746	721	663 (89)	450	439	430	427	356 (79)
51-53[b]	747	745	745	720	661** (88)	452	440	430	427	358** (79)
78	821	777	803	773	704 (86)	515	500	498	492	410 (80)
77-79[b]	822	777	798	768	689** (84)	520	500	497	491	406** (78)
104	768	727 (95)	755 (98)	718 (93)	689 (90)	551	514 (93)	503 (91)	516 (94)	420 (76)
102-104 [b]	761	691	746	743	695 (91)	551	529	485	525	417** (76)

TABLE 2. Selected mean body weights of male and female rats receiving B-1216 for up to 104 weeks

Week of study	Dietary concentration (ppm)									
	Males					Females				
	0	1	10	100	1000	0	1	10	100	1000
Body weight gain (g)[c]										
1–13	239	238	237	229	206 (86)	108	106	103	100	77 (71)
1–26	334	332	332	315	282 (84)	156	151	150	147	108 (69)
1–52	451	453	452	427	374 (83)	260	249	240	238	173 (67)
52–104	23	-21	9	-3	26	101	75	73	89	64 (63)
1–104	475	434	468	425	402 (85)	367	327	313	332	239 (65)

Data taken from pp. 31, Table 3 (pp. 52-55) and pages 56 and 57, MRID 42248620.
[a]Percent of control calculated by the reviewer.
[b]Statistical analysis performed on mean body weights for 3-week intervals.
[c]Weight gain calculated by the reviewer except for the interval of weeks 1-104.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Food consumption data for 13- or 26-week intervals are summarized in Table 3. Weekly food consumption values were slightly lower in 1000-ppm group male rats than controls throughout the study, but the differences between treated and controls did not exceed 8%. Weekly food consumption by the 1000-ppm group females was less than that of control throughout the study and the values ranged up to -18% at week 103. For each 13- or 26-week interval male rats consumed up to 6% ($p<0.01$) less food than the control rats and females consumed up to 12% less than the control rats. Overall food consumption for the entire study was reduced by 5% in 1000-ppm group males and by 9% in 1000-ppm group females compared with their respective controls.

2. Compound consumption

Compound intake is presented in Table 1. Males consumed an average of 0.04, 0.38, 3.82, and 40 mg B-1216/kg body weight/day and females consumed an average of 0.05, 0.47, 4.87, and 53 mg B-1216/kg/body weight/day at 1, 10, 100, and 1000 ppm, respectively.

3. Food efficiency

The study authors calculated the food utilization ratio (grams food consumed/g weight gained) and the reviewer calculated food efficiency ([g weight gained/g food consumed] × 100) (Table 3). Both methods showed that 1000-ppm group rats gained less weight for the food consumed than the control groups, indicating the reduced food consumption accounted for only part of the decreased body weights.

TABLE 3. Selected mean food consumption, food utilization and food efficiency values for male and female rats receiving B-1216 for up to 104 weeks

Week of study	Dietary concentration (ppm)									
	Males					Females				
	0	1	10	100	1000	0	1	10	100	1000
Food consumption (g/rat/13- or 26-week period)										
1–13	2243	2225	2244	2227	2100** (94)[a]	1596	1577	1556* (97)	1550* (97)	1475** (92)
14–26	2222	2211	2244	2206	2115** (95)	1579	1567	1564	1570	1475** (93)
27–52	4436	4426	4486	4429	4236** (95)	3296	3278	3214	3274	3048** (92)
53–78	4551	4414	4610	4503	4300* (94)	3623	3549	3453	3509	3291** (91)
79–104	4866	4774	4804	4714	4686 (96)	4113	3924	3792**	3804**	3633** (88)
1-104	18300	18093	18100	17924	17404 (95)	14105	13812	13503	13728	12887 (91)
Food utilization or food efficiency										
1–26[b]	11.6	11.7	11.7	12.0	12.8	18.0	18.3	18.4	18.8	24.2
1-26[c]	8.7	8.7	8.6	8.3	7.8	5.5	5.3	5.4	5.4	4.1
1-104[c]	2.60	2.40	2.59	2.37	2.31	2.60	2.37	2.32	2.42	1.85

Data taken from pp.31, 32, 50, and 51, MRID 42248620.
[a]Percent of control value calculated by the reviewer.
[b]Food utilization (g food consumed/g body weight gain)
[c]Food Efficiency ([g body weight gain/grams food consumed] × 100), calculated by the reviewer.

D. OPHTHALMOSCOPIC EXAMINATION

Eye examinations at weeks 26, 52, 78, and 101 of the study showed no effects related to treatment with the test material in 1000-ppm group animals compared with the controls.

E. BLOOD WORK

1. Hematology

Erythrocyte parameters are summarized in Table 4. Red blood cell (RBC) count, hematocrit, and hemoglobin concentrations were slightly reduced in 1000-ppm group male and female rats up to week 78 of the study. Statistical significance was achieved for some or all the parameters at each time point. RBC count was reduced by 9% in males and 4–8% in females, hematocrit by 2–7% in males and 3–8% in females, and hemoglobin by 5–12% in males and 5–8% in females. Erythrocyte cell volume (MCV) and corpuscular hemoglobin concentrations (MCHC) fluctuated and are not considered treatment related. These results suggest a mild anemia in both sexes at 1000 ppm. RBC counts, hematocrit, and/or hemoglobin concentrations in

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TABLE 4. Selected hematologic values in male and female rats fed B-1216 for up to 104 weeks										
Conc. (ppm)	Males					Females				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm	0 ppm	1 ppn	10 ppm	100 ppn	1000 ppm
Week 13										
Hct (%)	50.8	49.2	47.9* (94)	48.4* (95)	49.7* (98)	50.0	48.8	47.4* (95)	47.2** (94)	46.2** (92)
Hgb (g/dL)	15.3	14.9	14.7* (96)	14.8* (97)	14.4** (94)	15.4	14.9	14.6	14.5* (94)	14.2** (92)
RBC (× 10^6/mm^3)	7.40	7.45	7.45	7.38	7.46	7.12	6.88	6.94	6.82* (96)	6.69** (94)
MCHC	30.1	30.4	30.8	30.5	29.0	30.10	30.52	30.78	30.72	30.66
MCV (fL)	68.7	66.0*	64.4* (94)	65.5* (95)	66.7* (97)	70.3	70.9	68.5	69.2	69.0
Week 26										
Hct (%)	48.1	46.6* (97)	46.7* (97)	46.7* (97)	45.0** (94)	45.0	44.7	43.4	43.2* (96)	42.7** (95)
Hgb (g/dL)	15.43	14.86	15.05	15.08	14.72** (95)	15.0	14.7	14.4* (96)	14.7* (98)	14.3** (95)
RBC (× 10^6/mm^3)	8.10	7.97	8.12	8.14	8.31	7.73	7.88	7.32	7.48	7.11** (92)
MCHC	32.09	31.89	32.22	32.29	32.73	33.4	33.0	33.1	34.0	33.6
MCV (fL)	59.5	58.6	57.6	57.4	54.3** (91)	58.2	56.8	59.3	57.7	60.0
Week 52										
Hct (%)	49.4	48.7	49.8	47.4* (96)	45.8** (93)	45.2	47.7	45.2	44.5	43.8 (97)
Hgb (g/dL)	15.37	15.08	15.09	14.36** (93)	13.59** (88)	14.5	15.1	14.5	14.3	13.5* (93)
RBC (× 10^6/mm^3)	8.52	8.60	8.59	7.99* (94)	7.77** (91)	7.34	7.42	7.39	7.28	6.86* (96)
MCHC	31.1	31.0	30.3* (97)	30.3* (97)	29.7** (95)	32.2	31.8	32.0	32.0	30.9** (96)
MCV (fL)	58.1	56.8	58.1	59.3	59.1 (102)	61.7	64.0	61.4	61.2	63.9* (104)

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TABLE 4. Selected hematologic values in male and female rats fed B-1216 for up to 104 weeks										
Conc. (ppm)	Males					Females				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm	0 ppm	1 ppn	10 ppm	100 ppn	1000 ppm
Week 78										
Hct (%)	46.2	46.1	46.8	44.5	43.5 (94)	44.0	45.1	43.7	43.3	41.4* (94)
Hgb (g/dL)	14.3	14.0	14.2	13.3	12.7** (89)	13.7	14.1	13.7	13.5	12.8 (93)
RBC ($\times 10^6$/mm^3)	7.07	7.08	7.08	6.64	6.44* (91)	6.39	6.55	6.32	6.31	6.11 (96)
MCHC	31.0	30.3	30.2	29.9* (96)	29.1* (94)	31.04	31.33	31.20	31.16	30.89
MCV (fL)	65.5	65.3	66.3	67.2	67.7	68.9	68.9	69.8	68.6	67.9
Week 102										
Hct (%)	45.1	44.0	45.0	43.7	43.6	42.6	43.9	46.4	44.4	44.3
Hgb (g/dL)	13.75	13.57	13.75	13.26	13.09	13.39	13.86	14.42	13.70	13.83
RBC ($\times 10^6$/mm^3)	6.48	6.40	6.45	6.34	6.45	5.95	6.18	6.55	6.25	6.28
MCHC	30.46	30.89	30.52	30.32	30.01	31.52	31.58	31.08	30.86	31.22
MCV (fL)	69.8	69.0	69.6	69.0	67.7	72.2	71.0	70.9	71.2	70.6

[a] Data taken from Table 7, pp. 67-76, MRID 42248620.

*p≤0.05, **p≤0.01, treated group compared with control.

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100-ppm group rats were significantly less than that of controls up to week 52 for males and week 26 for females. Except for a 7% decrease in hemoglobin in 100–ppm group male rats at week 52, the changes were within 5 or 6% of control values for both sexes and are not considered biologically significant. Sporadic statistically significant changes were observed at 1 and 10 ppm, but were not considered treatment related.

2. Clinical chemistry

Selected serum chemistry values are summarized in Table 5. Statistically significant changes in serum chemistry values were observed for both sexes. Although the serum protein content showed no statistically significant differences between treated and control groups, serum albumin levels were significantly ($p<0.01$ or <0.05) elevated by 3–6% and globulin levels decreased by 9–11% at weeks 13-52 in 1000-ppm group males compared with controls. Serum albumin and globulin levels in 1000-ppm group females (+8 and -5%, respectively) were also significantly different ($p<0.05$) from control values, but only at week 13. Male rats administered 10, 100, or 1000 ppm of the test material had significantly higher serum creatinine levels (+16 to +27%, $p<0.01$ or <0.05) at weeks 13 and 52 and males administered 1000 ppm also had higher levels at 26 weeks (+17%, $p<0.05$). Creatinine levels were significantly higher that than that of controls in the 1-, 10-, and 100-ppm group females at week 26 (+20 to + 44%), the 100-ppm group at weeks 78 and 102 (+32% and +14%, $p<0.05$), and the 1000-ppm group at weeks 52, 78, and 102 (+11%, +39%, and +5%, respectively, $p<0.01$ or <0.05). Male and female rats in the 1000-ppm dose groups had higher serum cholesterol levels than controls throughout the study. The levels in the males were statistically significant at weeks 26 (+39%) and 52 (+32%), but not at weeks 13, 78, and 102 (5% to 29%). The levels in the females were statistically significant at weeks 13 and 52-102 (+24% to +115%) but not at week 26 (+25%). Cholesterol levels were also consistently elevated throughout the study at 100 ppm in males by +13% to +44% and in females by +6% to 48%; statistical significance was achieved at week 52 for both sexes. Changes in other parameters showing statistically significant differences between treated and control groups were very small in magnitude or showed no dose-related trends and are, consequently, considered biologically insignificant or unrelated to treatment with the test material.

F. URINALYSIS

Protein was detected in the urine of all male rats sampled during the study. Urine protein levels were significantly ($p<0.05$) increased in male rats administered 1000 ppm of the test material for 12 weeks (+16%) and 25 weeks (+114%). The protein content in treated female rats at week 101 was significantly lower than that of controls at all dose levels. No clear dose-related trend was observed for either sex. The urine of 1000-ppm group males was significantly ($p<0.01$) more acidic (pH 6.80) than that of control (pH 7.63) at week 51. No other statistically significant changes were observed in male or female rats treated with the test material as compared with the control rats.

TABLE 5. Selected serum chemistry values in male and female rats fed B-1216 for up to 104 weeks

Conc. (ppm)	Males					Females				
	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm	0 ppm	1 ppn	10 ppm	100 ppn	1000 ppm
Week 13										
Albumin (g/dL)	3.88	3.69	3.77	3.90	4.11*	4.25	4.33	4.37	4.37	4.60*
Globulin (g/dL)	3.40	3.34	3.21	3.11	3.07*	3.30	3.00*	3.05*	3.96*	3.13*
Creat. (mg/dL)	0.57	0.65	0.72*	0.66*	0.66*	0.64	0.73	0.70	0.75	0.67
Cholest. (mg/dL)	61	56	59	69	73	76	79	68	76	94**
Week 26										
Albumin (g/dL)	3.61	3.65	3.63	3.62	3.73**	4.33	4.64	4.43	4.57	4.54
Globulin (g/dL)	3.81	3.71	3.49	3.56	3.39*	3.37	3.30	3.29	3.34	3.31
Creat. (mg/dL)	0.54	0.52	0.57	0.53	0.63*	0.45	0.65**	0.60**	0.54**	0.46**
Cholest. (mg/dL)	56	55	58	70	78*	77	88	74	82	96
Week 52										
Albumin (g/dL)	4.01	3.98	3.98	3.98	4.23*	4.83	4.88	4.07	4.67	4.84
Globulin (g/dL)	3.37	3.31	3.18	3.26	3.06*	3.19	3.08	3.19	3.27	3.28
Creat. (mg/dL)	0.56	0.63	0.69**	0.68**	0.71**	0.66	0.66	0.65	0.72	0.73*
Cholest. (mg/dL)	72	76	83	104*	95*	86	87	87	123*	130**
Week 78										
Albumin (g/dL)	3.96	3.87	4.19	4.01	4.05	4.53	4.46	4.58	4.37	4.39
Globulin (g/dL)	3.61	3.59	3.29	3.55	3.46	3.44	3.38	3.60	3.21	3.53
Creat. (mg/dL)	0.59	0.72	0.69	0.85	0.84	0.59	0.67	0.69	0.78*	0.82**
Cholest. (mg/dL)	101	105	107	125	130	98	90	92	145	153*
Week 102										
Albumin (g/dL)	3.63	3.74	3.71	3.79*	3.94*	4.26	4.35	4.50	4.31	4.37
Globulin (g/dL)	3.75	3.65	3.38	3.25*	3.56*	3.66	3.38	3.48	3.32	3.42
Creat. (mg/dL)	0.81	0.70	0.80	1.04	0.71	0.65	0.68	0.67	0.74*	0.68*
Cholest. (mg/dL)	135	116	159	162	142	101	107	116	129	217*

Data taken from Table 8, pp. 77-86, MRID 42248620.
*p<0.05, p<0.01, statistically significant, treated groups compared with controls.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Selected organ weights are presented in Table 6. At the 52-week interim sacrifice, absolute thyroid and liver weights of 1000-ppm group males exceeded that of controls by 43% (p<0.01) and 27% (0<0.05), respectively, and the relative weights were 64% and 45% greater than that of the control. Absolute and relative weights of the liver in 1000-ppm group females exceeded that of control rats by 22% (p<0.01) and 54%,

respectively and in 100-ppm group females by 16% ($p<0.05$) and 29%, respectively. The mean terminal body weight of the 1000-ppm group females was also significantly less than that of controls at 52 weeks.

At study termination, the absolute and relative heart weights were significantly reduced by 15 and 9%, respectively, and the absolute and relative kidney weights were reduced by about 25% ($p<0.01$) and 19%, respectively, in 1000-ppm group male rats compared with the organ weights in controls. The liver weight in 1000-ppm group male rats exceeded that of controls by only 17% (N.S.). In 1000-ppm group female rats, the mean terminal body weight was reduced by 24% ($p<0.01$); the mean absolute and relative liver weights exceeded that of controls by 24% ($p<0.01$) and 63%, respectively. Absolute and relative pituitary weights in 1000-ppm group females were markedly less than that of controls (-75 and -65%, respectively).'

TABLE 6. Selected organ weights in male and female rats fed B1216 for up to 104 weeks

Organ	Dietary Concentration (ppm)				
	0	1	10	100	1000
Male – 52-weeks					
Terminal body weight (g)	757	790	741	731	663
Thyroid (g)	29.6 (3.91)[a]	31.7	27.2	31.0	42.4** (6.40)
Liver (g)	27.7 (3.66)	29.7	26.2	26.7	35.2* (5.31)
Female – 52 weeks					
Terminal body weight (g)	491	484	452	440	387**
Liver (g)	15.8 (3.22)	16.4	15.7	18.3* (4.16)	19.2** (4.96)
Males –104 weeks					
Terminal body weight (g)	738	700	716	698	678
Heart (g)	2.41 (0.33)	2.21	2.18	2.24	2.05** (0.30)
Liver (g)	27.9 (3.78)	27.5	28.0	27.0	29.9 (4.41)
Kidney (g)	7.91 (1.07)	7.00	7.40	6.63	5.92** (0.87)
Females – 104 Weeks					
Terminal body weight (g)	542	502	480	499	413**
Pituitary (g)	80.0 (14.76)	94.6	80.2	66.3	21.6* (5.23)
Liver (g)	18.5 (3.41)	17.5	18.5	20.4	23.0** (5.57)

Data taken from Table 10, pp. 92-95, MRID 42248620.
[a]Relative organ weights ([organ weight (g)/terminal body weight (g)] × 100) in parentheses, calculated by the reviewer
*$p<0.05$, **$p<0.01$, statistically significant, treated groups compared with controls.

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2. Gross pathology

Yellow/brown stained fur was the only gross finding that occurred at a significantly higher incidence in male and female rats fed the1000-ppm diet for 52 weeks when compared with the controls. Table 7 summarizes the notable gross findings in rats fed the test material for up to 104 weeks. Gross lesions were found at significantly increased incidences in 1000-ppm group animals compared with that of controls in the liver, lungs, and fur of both sexes, testes and thyroid of males, and kidneys and skin of females. In 1000-ppm group male rats, the incidences were 46% for swollen liver (24% in controls, $p<0.05$), 14% for accentuated lobular markings in the liver (0% for controls, $p<0.01$), 12% for lung petechia (0% for control, $p<0.01$), 24% for flaccid testes (10% for control, $p=0.054$), 14% for white subtunical striae in the testes (2% for control, $p<0.05$), 18% for enlarged thyroid (4% for control, $p<0.05$), and 26% for general yellow staining of the fur (0% for control, $p<0.01$). In 1000-ppm group females, the incidences were 14% for enlarged (0% for control, $p<0.01$), 24% for pitted (0% for control, $p<0.01$), and 14% for mottled livers (2% for control, $p<0.05$), 12% for lung petechiae (2% for control, $p=0.056$), 24% for pale kidney (6% for control, $p<0.01$), 44% for patchy alopecia of the skin (16% for control, $p<0.01$), and 66% for general yellow-brown staining of the skin (0% for control, $p<0.01$). In addition, the incidence of white subtunical striae of the testes also was marginally increased to12% ($p=0.056$) in 100-ppm group males, and the incidence of patchy alopecia was significantly increased to 32% ($p<0.05$) in 100-ppm group females. The remaining gross findings occurred with similar incidences in treated and control animals.

TABLE 7. Notable gross findings in male and female rats fed B1216 for up to 104 weeks

Gross lesion/organ	Dietary concentration (ppm)				
	0	1	10	100	1000
Males					
No. animals examined	50	50	50	50	50
Liver					
Swollen	12	14	18	15	23*
Lobular markings accentuated	0	1	4	2	7**
Lungs					
Petechia	0	2	0	1	6*
Testes					
Flaccid	5	6	5	8	12†
White subtunical striae	1	6	0	6†	7*
Thyroid					
Enlarged	2	2	4	4	9*
Fur					
General yellow stain	0	0	0	0	13**

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TABLE 7. Notable gross findings in male and female rats fed B1216 for up to 104 weeks

Gross lesion/organ	Dietary concentration (ppm)				
	Females				
No. animals examined	50	50	50	50	50
Liver					
Enlarged	0	1	0	0	7**
Pitted	0	1	0	0	12**
Mottled	1	0	1	2	7*
Lungs					
Petechia	1	1	2	2	6†
Kidneys					
Pale	3	4	7	7	12**
Skin					
Patchy alopecia	8	14	11	16*	22**
Fur					
General yellow/brown stain	0	0	0	0	33**

Data taken from Table 12, pp. 101-112, MRID 42248620.
†p=0.054-0.056, *p<0.05, **p<0.01, statistically significant, treated groups compared with controls.

3. Microscopic pathology

a) Non-neoplastic – Notable microscopic findings are summarized in Tables 8 (males) and 9 (females). Treatment-related lesions occurred in the liver, pancreas, and lungs of both sexes; the kidney, testes, and adrenal gland of male rats; and lymph nodes of female rats. Possibly treatment-related lesions were also observed in the thyroid of both sexes

At the 52-week interim sacrifice, the incidence of lung pneumonitis was increased in males receiving the 1000-ppm diet. In the pancreas, acinar epithelial vacuolation occurred in eight females at 1000 ppm but in none at the lower doses or in controls; exocrine degranulation occurred in seven females at 1000 ppm, three at 100 ppm one at 10 ppm, and in none at 1 ppm or in controls; and exocrine atrophy occurred in four males at 1000 ppm and in two controls, but no increase in severity was observed. In the liver, centrilobular hepatocyte vacuolation was observed in five to 11 males in all groups including controls and in four to seven females in all groups except controls; no clear-dose response relationship was observed for either sex, but the severity showed a dose-related increase. Centrilobular fat was observed in the liver of four to ten male rats in each group including controls and in three to seven females in the four treatment groups; the severity in treated males was greater than that of controls in all treated groups.

Table 8. Notable nonneoplastic microscopic findings in male rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)				
	0	1	10	100	1000
Males – 52 Week Interim Sacrifice					
Lungs, No. animals examined	10	9	10	10	10
Pneumonitis, minimal	0	0	1	1	7
Liver, No. animals examined	10	10	10	10	10
Centrilobular hepatocyte vacuolation	5 (2.20)[a]	8 (2.38)	5 (2.40)	7 (2.57)	11 (3.00)
Centrilobular fat	5 (1.60)	6 (2.17	4 (2.25)	5 (2.20)	10 (2.90)
Pancreas, No. animals examined	10	10	10	10	10
Acinar epithelial vacuolation	0	0	0	0	0
Exocrine degranulation, minimal	0	0	0	1	0
Exocrine atrophy	2 (3.00)	0	0	0	4 (2.00)
Males – Main Study Group					
Lungs, No. animals examined	50	50	50	50	50
Alveolar adenomatosis	0	0	0	0	4† (2.00)
Pneumonitis	1	1	1	3	8* (2.13)
Alveolar epithelialization	0	0	1	0	17** (2.12)
Aggregates of alveolar macrophages	0	0	2	2	3
Lymph Nodes, No. Animals examined	50	50	50	50	50
Sinus histiocytosis	8	5	4	4	7
Liver, No. animals examined	50	50	50	50	50
Eosinophilic hepatocytes	9	6	7	6	24**
Centrilobular hepatocyte rarefaction and vacuolation	0	2	0	4† (2.00)	19** (2.58)
Centrilobular hepatocyte vacuolation	7 (2.00)	7 (2.00)	6 (2.17)	9 (2.33)	20** (2.16)
Centrilobular hepatocyte necrosis	1	1	0	0	0
Centrilobular sinusoidal dilatation	0	2	1	5*	7**
Centrilobular fat[b]	0	2 (1.5)	3 (2.33)	3 (2.00)	18** (2.61)
Bile duct hyperplasia	21 (2.29)	15 (2.40)	19 (2.21)	17 (2.29)	31* (2.65)
Pericholangitis	2 (2.50)	3 (2.00)	1 (2.00)	9* (2.11)	12** (2.17)
Pancreas, No. animals examined	50	48	50	50	50
Acinar epithelial vacuolation	0	1	1	0	1
Exocrine atrophy	7 (2.29)	11 (2.55)	9 (2.33)	13 (2.31)	19** (2.59)
Acinar epithelial fat	0	0	0	0	0
Kidney, No. animals examined	50	50	50	50	50
Cortical tubular basophilia	3 (2.33)	8 (2.25)	9 (2.33)	7 (2.43)	11* (2.55)
Adrenal gland, No. animals examined	50	48	44	40	50
Cortical cystic degeneration	0	0	2 (3.00)	4† (2.50)	4† (2.25)
Thyroid gland, No. animals examined	50	35	40	35	50
Follicular hyperplasia	1	2	1	0	4
Testes, No. animals examined	50	50	50	49	50
Atrophy	7 (3.14)	15* (2.93)	9 (3.00)	19** (3.53)	20** (3.70)
Spermatocele granuloma	0	1	1	0	5*

Data taken from Tables 14 (pp. 121-128) and 17 (pp. 151-166), MRID 42248620.
[a]The numbers in parentheses are severity grades: 1 = slight, 2 = minimal, 3 = moderate, 4 = marked.
[b]Number of animals examined: 50, 32, 34, 28, and 50 in each dose group respectively.
†p=0.055–0.059, *p<0.05, **p<0.01, statistically significant, treated group compared with controls, calculated by the review using Fisher's Exact Test.; N denotes a negative trend.

TABLE 9. Notable nonneoplastic microscopic findings in female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)				
	0	1	10	100	1000
Females – 52 Week Interim Sacrifice					
Lungs, No. animals examined	10	10	10	10	10
Pneumonitis, minimal	1	0	0	0	2
Liver, No. animals examined	10	10	10	10	10
Centrilobular hepatocyte vacuolation	0	4 (2.00)[a]	6 (2.33)	7 (2.45)	6 (2.67)
Centrilobular fat	0	3 (2.20)	7 (2.14)	7 (2.29)	5 (2.22)
Pancreas, No. animals examined	10	10	10	10	10
Acinar epithilial vacuolation	0	0	0	0	8 (2.13)
Exocrine degranulation, minimal	0	0	1	3	7
Exocrine atrophy	1 (2.00)	1 (2.00)	1 (2.00)	2 (2.50)	1 (2.00)
Females – Main Study Group					
Lungs, No. animals examined	50	50	50	50	50
Alveolar adenomatosis	1	0	0	0	0
Pneumonitis	1	0	1	4	5
Alveolar epithelialization	0	0	1 (2.00)	4† (2.25)	15** (2.47)
Aggregates of alveolar macrophages	0	2	0	1	5*
Lymph Nodes, No. animals examined	50	50	50	50	50
Sinus histiocytosis	4 (2.50)	5 (2.22)	8 (2.38)	3 (2.33)	18** (2.56)
Liver, No. animals examined	50	50	50	50	50
Eosinophilic hepatocytes	4	9	9	11*	34**
Centrilobular hepatocyte rarefaction and vacuolation	0	1 (3.00)	0	4† (2.25)	29** (2.83)
Centrilobular hepatocyte vacuolation	18 (2.33)	11 (2.45)	10 (2.30)	19 (2.53)	9*[N] (2.44)
Centrilobular hepatocyte necrosis	1 (2.00)	0	1 (3.00)	1 (2.00)	7* (2.57)
Centrilobular sinusoidal dilatation	1	0	1	9**	28**
Centrilobular fat[b]	6 (2.00)	1 (2.00)	3 (2.67)	3 (2.67)	20** (2.65)
Bile duct hyperplasia	15 (2.20)	14 (2.36)	14 (2.14)	20 (2.50)	38* (2.47)
Pericholangitis	1 (2.00)	0	1 (2/00)	7* (2.14)	12** (2.00)
Pancreas, No. animals examined	50	50	50	50	50
Acinar epithelial vacuolation	0	0	1 (2.00)	3 (2.00)	28** (2.32)
Exocrine atrophy	3 (2.33)	6 (2.5)	3 (2.33)	13** (2.69)	13** (2.62)
Acinar epithelial fat[c]	0	1 (2.00)	0	1 (2.00)	16** (2.31)
Kidney, No. animals examined	50	50	50	50	50
Cortical tubular basophilia	4	4	4	5	3
Adrenal gland, No. animals examined	50	43	45	46	50
Cortical cystic degeneration	14	12	18	16	12
Thyroid gland, No. animals examined	50	25	27	32	50
Follicular hyperplasia	1	0	0	0	5

Data taken from Tables 14 (pp. 129-136) and 17 (pp. 167-182), MRID 42248620.
[a]The numbers in parentheses are severity grades: 1 = slight, 2 = minimal, 3 = moderate, 4 = marked.
[b]Number of animals examined: 50, 25, 26, 29, and 50 in each dose group, respectively.
[c]Number of animals examined: 18, 25, 24, 21, and 30 in each group, respectively.
†p=0.055–0.059, *p<0.05, **p<0.01, statistically significant, treated group compared with controls, calculated by the review using Fisher's Exact Test.

In main study rats, the incidences of numerous liver lesions were significantly ($p<0.01$ or <0.05) increased in male and female rats fed the 1000-ppm diet, and the incidences of a few liver lesions were significantly ($p<0.01$ or <0.05) or marginally ($p=0.055$-0.059) increased in both sexes administered the 100-ppm diets. Liver lesions included eosinophilic hepatocytes, centrilobular hepatocyte rarefaction and vacuolation, centrilobular hepatocyte vacuolation, centrilobular hepatocyte necrosis, centrilobular sinusoidal dilatation, and centrilobular fat. Except for hepatocyte necrosis (incidence not increased in males), these lesions were seen in 48, 38, 40, 14, and 36%, respectively, of 1000-ppm group male rats compared with 18, 0, 14, 0, and 0%, respectively, of controls. Except for centrilobular hepatocyte vacuolation (incidence not increased in females), the lesions occurred in 68, 58, 14, 56, and 40%, respectively, of 1000-ppm group females compared with 8, 0, 2, 2, and 12%, respectively, of controls. Centrilobular hepatocyte vacuolation occurred in significantly fewer females at 1000 ppm than in the control group. The incidences of bile duct hyperplasia and pericholangitis, respectively, were significantly increased in 1000-ppm group male (62 and 24%) and female rats (76 and 24%) compared with the control incidences of 42 and 4%, respectively, for males and 30 and 2%, respectively, for females. At 100 ppm, the incidence of centrilobular hepatocyte vacuolation (8%) was marginally increased and sinusoidal dilatation (10%) and pericholangitis (18%) were significantly increased in males. In females receiving the 100-ppm diet, the incidences of eosinophilic hepatocytes (22%), centrilobular sinusoidal dilatation (18%), and pericholangitis (14%) were significantly increased and the incidence of centrilobular hepatocyte rarefaction and vacuolation (8%) was marginally increased compared with control incidences.

Microscopic findings in the lungs consisted of alveolar epithelialization in 34% ($p<0.01$) of males and 30% ($p<0.01$) of females, pneumonitis in 16% ($p<0.05$) and alveolar adenomatosis in 8% ($p=0.059$) of males, and aggregates of alveolar macrophages in 10% of females. Alveolar epithelialization also occurred in 8% ($p=0.059$) of 100-ppm group female rats. None of these lesions occurred in control animals except for pneumonitis in one control male and one control female.

An increased incidence of thyroid follicular hyperplasia was observed in males at 1000 ppm (8% vs 2% for controls) and in females at 1000 ppm (10% vs 2% in controls).

Males administered the 1000-ppm diet had a significantly increased incidence of exocrine atrophy of the pancreas (38% vs 14% for controls); the incidence for 100-ppm group males was 26% but was not statistically significant. Male rats administered the 1000-ppm diet also had significantly increased incidences of cortical tubular basophilia (22% vs 6% for controls) in the kidney and testicular spermatocele granuloma (10% vs 0% for controls). Testicular atrophy occurred in 30% ($p<0.05$), 39% ($p<0.01$) and 40% ($p<0.01$) of male rats administered the 1, 100, and 1000-ppm diets, respectively, compared with 14% of controls. A

marginally significant increase of 8% (p=0.059) was seen for the incidence of cortical cystic degeneration in the adrenal gland of 100- and 1000-ppm group males. The incidence of sinus histiocytosis in lymph nodes was significantly increased in 1000-ppm group females (36%) compared with controls (8%). Females administered 1000 ppm had significantly ($p<0.01$) increased incidences of acinal epithelial vacuolation (56% vs 0% for control), exocrine atrophy (26% vs 6% for control), and acinar epithelial fat (32% vs 0% for control) in the pancreas. Exocrine atrophy also occurred in 26% ($p< 0.01$) of 100-ppm group females.

Assessment of the additional sections of the brain (cerebrum, cerebellum/pons/ medulla/midbrain) and cervical spinal cord from male and female rats in the control and 1000-ppm dose groups showed no treatment-related effect on the incidence or the severity of vacuolation of white matter. White matter vacuolation graded trace or minimum was observed in large numbers of animals for all groups including controls (52 to 84% for males and 74 to 94% for females).

b) Neoplastic –

There were no neoplasms of concern identified in the satellite (52 week interim sacrifice) animals.

A slightly increased incidence of thyroid gland follicular cell adenomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 100 ppm and 1000 ppm. The incidence was 8%, 6%, 10%, 14% and 14% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 13% and the mean was 6.5%. Also, a slightly increased incidence of thyroid gland follicular cell adenocarcinomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 1000 ppm. The incidence was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 5% and the mean was 1.4%. In addition, slightly increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats, but these incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). Further, this type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in males and females and of mammary gland fibroadenomas in females were also observed in all groups, including controls. These neoplasms are not considered to be treatment-related. A noteworthy increase of tumors was not observed in any other tissues in the treated male or female rats in this study.

Table 10. Selected neoplastic microscopic findings in male rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)				
	0	1	10	100	1000
Males – Main Study Group					
Total no. of tumors	43	46	42	40	42
Lungs, No. animals examined	50	50	50	50	50
Pulmonary adenoma	0	0	0	0	1
Pulmonary adenocarcinoma	0	0	0	0	1
Combined	0	0	0	0	2
Liver, No. animals examined	50	50	50	50	50
Benign liver cell tumors	0	0	1	0	3
Malignant liver cell tumors	3	1	0	0	1
Combined	3	1	1	0	4
Pancreas, No. animals examined	50	48	50	50	50
Islet cell adenoma	4 (8%)	5 (10%)	12 (24%)	7 (14%)	9 (18%)
Islet cell adenocarcinoma	1 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Combined	5	5	12	7	9
Adrenal gland, No. animals examined	50	48	44	40	50
Cortical adenoma	0	1	0	0	0
Cortical carcinoma	0	0	0	0	0
Combined	0	1	0	0	0
Thyroid gland, No. animals examined	50	35	40	35	50
Follicular adenoma	4 (8%)	2 (6%)	4 (10%)	5 (14%)	7 (14%)
Follicular adenocarcinoma	0 (0%)	0 (0%)	0 (0%)	1 (3%)	3 (6%)
Combined	4	2	4	6	10
Pituitary gland, No. animals examined	50	42	42	37	50
Pituitary adenoma	30	29	30	22	24
Pituitary adenocarcinoma	1	0	0	0	0
Combined	31	29	30	22	24
Testes, No. animals examined	50	50	50	49	50
Interstitial cell tumor	3	5	3	7	4

Data taken from Table 16 (pp. 138-144), MRID 42248620.

Historical Control Data for Male Rats (From MRID 45150201)

Twenty studies performed at Huntingdon Life Sciences, Huntingdon, England; started between 1981 and 1990; studies of 101-111 weeks duration.

Thyroid Gland (Males)

Follicular cell adenomas	Range	0 - 13%
	Mean	6.5%
Follicular cell adenocarcinomas	Range	0 - 5%
	Mean	1.4%

Pancreas (Males)

Islet cell adenomas	Range	0 - 28%
	Mean	13.8%
Islet cell adenocarcinomas	Range	0 - 12%
	Mean	4.0%

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Table 11. Selected neoplastic microscopic findings in female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)				
	0	1	10	100	1000
Females – Main Study Group					
Total no. of tumors	44	45	43	47	39
Lungs, No. animals examined	50	50	50	50	50
Pulmonary adenoma	0	0	0	1	0
Pulmonary adenocarcinoma	0	0	0	0	0
Combined	0	0	0	1	0
Liver, No. animals examined	50	50	50	50	50
Benign liver cell tumors	0	0	1	0	0
Malignant liver cell tumors	0	0	0	0	0
Combined	0	0	1	0	0
Pancreas, No. animals examined	50	50	50	50	50
Islet cell adenoma	4	5	3	3	0
Islet cell adenocarcinoma	0	0	0	0	0
Combined	4	5	3	3	0
Adrenal gland, No. animals examined	50	43	45	46	50
Cortical adenoma	1	1	0	0	0
Cortical carcinoma	0	0	0	1	3
Combined	1	1	0	1	3
Thyroid gland, No. animals examined	50	25	27	32	50
Follicular adenoma	1	0	0	0	2
Follicular adenocarcinoma	0	0	0	1	0
Combined	1	0	0	1	2
Pituitary gland, No. animals examined	50	43	41	45	50
Pituitary adenoma	34	37	33	38	26
Pituitary adenocarcinoma	2	2	3	0	1
Combined	36	39	36	38	27
Mammary gland, No. animals examined	23	26	24	32	17
Mammary fibroadenoma	20	24	19	27	16

Data taken from Table 16 (pp. 145-150), MRID 42248620.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study authors concluded that no tumorigenic potential was observed in male or female rats fed the test material, B-1216, at dietary concentrations 1-1000 ppm for up to 104 weeks. Treatment-related toxicity was observed in both sexes at 100 and 1000 ppm manifested by decreases in body weight gain, food consumption, food utilization, slight anemia, slight elevation in serum cholesterol, increased liver weight, increased numbers of animals with macroscopic liver or testicular lesions and increased numbers of animals with microscopic lesions in the liver, lungs, pancreas, lymph node and testis. The study author concluded that the no-observed-effect level (NOEL) was 10 ppm.

B. REVIEWER'S DISCUSSION/CONCLUSIONS

No treatment-related effects were observed on mortality; sufficient animals survived to study termination for assessment of late-developing lesions. Clinical signs of toxicity were limited to straw-discoloration of the fur in male and female rats and alopecia in female rats fed 1000 ppm of B-1216 for up to 104 weeks. Males and females administered the 1000-ppm diet showed statistically significant decreases in body weight throughout most of the study with final body weight decreased by 10% in males and 24% in females. The greatest effect on body weight gain occurred during the first year of the study, and overall weight gain was reduced by 15 and 35% for male and female rats, respectively. Reductions in food consumption were observed in both sexes fed the 1000-ppm diet compared with that of controls; the overall reduction observed in females (9%) was greater than that observed in males (5%). In addition the amount of reduction in food consumption did not correspond to the decrease in body weight gain as reflected by the increase in the food utilization factor or the decrease in the food efficiency values. These values suggested that decreases in weight gain were due in part to toxicity of the test material.

Hematologic effects in rats treated with the test material were manifested as a slight normochromic normocytic anemia at the 1000-ppm dietary level in both sexes. Clinical chemistry analyses showed that cholesterol levels were consistently elevated (frequently statistically significant) in both sexes at the 1000-ppm dietary level throughout the study. At 100 ppm, statistical significance was achieved only at week 52, but the increases occurred at each time point except week 13 for females. The cholesterol levels for treated animals were within the normal range for rats and the control values were in the lower range of the normal values. Nevertheless, the elevated cholesterol levels may have been related to liver toxicity. The statistically significant changes in globulin and albumin levels in 1000-ppm group male and female rats were of small magnitude compared with controls and not toxicologic significant. In addition, there was no treatment-related change in total protein, further verifying the lack of toxicologic significance. Creatinine levels were also elevated in male and female rats administered the test material, but the increases were small and the values were within the range of normal values.

Postmortem studies showed changes in organ weights at interim and terminal sacrifice. Absolute mean liver weights were significantly elevated in 1000-ppm group male and female rats at interim sacrifice and in 1000-ppm group females at terminal sacrifice. The small increase in 1000-ppm group males at terminal sacrifice was not statistically significant. The increased liver weight corresponded with gross and microscopic findings in the liver. The thyroid weight was increased at interim sacrifice and heart and kidney weights were decreased at terminal sacrifice in 1000-ppm group males; however, there were no gross or microscopic findings associated with the decreased heart weights. The increased thyroid weight corresponded with enlarged thyroids observed grossly in the 1000-ppm group males and with a slightly increased incidence of follicular cell hyperplasia and of follicular cell adenomas also in the 1000-ppm group males. Cortical basophilia was observed in the kidney of 1000-ppm group males, but it is unlikely that this finding was associated with the change in kidney weight. Female rats administered the 1000-ppm diet had decreased mean pituitary weight that may have been associated with the small decrease in the incidence of pituitary adenomas (52%) compared with that of controls (68%).

Gross lesions occurred with significantly increased incidences in the liver, lungs, testes, thyroid, and fur in 1000-ppm group males and in the liver, lungs, kidneys, skin, and fur of females. The gross lesions in the liver, lungs, testes, and possibly thyroid were likely associated with microscopic findings. No associated microscopic findings were associated with pale kidney in females and this is not considered treatment related. The yellow brown staining of the fur observed in males and females at 1000-ppm was likely associated with the test material, which was yellow in color. Alopecia in the females was observed at a significantly increased incidence in 100- and 1000-ppm group females. This finding is considered treatment related, but is of doubtful toxicologic significance in the absence of treatment-related microscopic skin lesions.

Treatment-related microscopic findings were observed in the liver, pancreas, lungs, kidneys, testes, and possibly thyroid gland.

Lesions in the liver (centrilobular hepatocyte vacuolation and centrilobular fat) were observed at all doses in females and in all 1000-ppm group males sacrificed at 52 weeks. In the main study groups, the incidences of eosinophilic foci, centrilobular hepatocyte rarefaction and vacuolation, hepatocyte vacuolation (males only), hepatocyte necrosis (females only), centrilobular fat, sinusoidal dilatation, bile duct hyperplasia, and pericholangitis were significantly elevated in the 1000-ppm males and females compared with control incidences. In addition, some of these lesions were significantly or marginally elevated in the 100 ppm males and females: eosinophilic hepatocyte (females only), hepatocyte rarefaction and vacuolation, sinusoidal dilatation, and pericholangitis.

Pancreatic lesions, exocrine atrophy in males and acinar epithelial vacuolation and exocrine degranulation in females, were observed in 1000-ppm group rats sacrificed at 52 weeks. In the main study groups, the incidence of exocrine atrophy remained significantly elevated in 1000-ppm group males, but the increased incidence noted at 100 ppm did not reach statistical significance. The incidence of exocrine degranulation was

not significantly increased in main study group females administered the1000-ppm diet. However, the incidence of acinar epithelial vacuolation remained elevated and the incidences of exocrine atrophy and acinar epithelial fat were increased at 1000 ppm. The incidence of exocrine atrophy was also increased at 100 ppm in main study females.

Pneumonitis was observed in most of the 1000-ppm group males at interim sacrifice. Eight male rats in the main study also had this lesion. Only one male control in both the interim sacrifice and main study groups had this lesion. Additional findings in main study male rats included alveolar adenomatosis, which was marginally increased at 1000 ppm, and alveolar epithelialization, which was significantly increased compared with the control incidence. No notable lung lesions occurred in female rats at interim sacrifice. However, the incidence of alveolar epithelialization was significantly increased in 1000-ppm group females and marginally increased in 100-ppm group females compared with the control incidence. In addition a small number of 1000-ppm group females had aggregates of alveolar macrophages in their lungs; this finding was not noted in the lungs of controls.

Kidney findings consisted only of an increased incidence of cortical tubular basophilia of the kidney in 1000-ppm group males in the main study. Treatment-related testicular lesions were not observed at 52 weeks; in the main study group treatment-related testicular lesions consisted of atrophy in males at 100 and 1000 ppm and a spermatocele granuloma in males at 1000 ppm. A significant increase in the incidence of testicular atrophy at 1 ppm was not considered treatment related, because there was no significant increase at the next higher dose of 10 ppm. The increased incidence of cortical cystic degeneration in the adrenal gland in 100- and 1000-ppm group males was marginal and no increase occurred between the two doses. Consequently, this lesion is not considered treatment related in the male rats. Female rats had an increased incidence of sinus histiocytosis in the lymph nodes at 1000 ppm in main study group, but not in the interim sacrifice study.

An increased incidence of thyroid follicular hyperplasia was observed in males at 1000 ppm (8% vs 2% for controls) and in females at 1000 ppm (10% vs 2% in controls). This finding may possibly be related to treatment with the test material.

In a separate report (dated 3/3/99, MRID 44807223), the investigators reported the results of a subsequent detailed histopathologic examination to evaluate the effect of B-1216 on white matter vacuolation of the brain and spinal cord in the male and female rats in the control and 1000-ppm groups in this study. This lesion was found in a long-term study of mice fed 100-7000 ppm of B-1216 for 2 years (MRID 42208405). In the rats in this present study, no toxicologically significant increase in the incidence of white matter vacuolation was observed in the brain or spinal cord. This lesion (white matter vacuolation of the brain and spinal cord) was observed at high incidences in all groups including controls, but the severity for all lesions was trace to minimum (i.e. severity was not increased in treated rats).

In conclusion, the lowest-observed-adverse-effect level (LOAEL) for B-1216 was 100 ppm for male and female rats based on liver toxicity in both sexes, testicular toxicity in males and pancreatic toxicity in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm.

A slightly increased incidence of thyroid gland follicular cell adenomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 100 ppm and 1000 ppm. The incidence was 8%, 6%, 10%, 14% and 14% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 13% and the mean was 6.5%. Also, a slightly increased incidence of thyroid gland follicular cell adenocarcinomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 1000 ppm. The incidence was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 5% and the mean was 1.4%. In addition, slightly increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats, but these incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). Further, this type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in males and females and of mammary gland fibroadenomas in females were also observed in all groups, including controls. These neoplasms are not considered to be treatment-related. A noteworthy increase of tumors was not observed in any other tissues in the treated male or female rats in this study.

This combined chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies guideline requirements for a chronic toxicity/carcinogenicity study [OPPTS 870.4300 (§83-5)] in rats.

C. STUDY DEFICIENCIES

The study authors did not summarize the data for clinical signs and they did not provide statistical evaluation of histopathology data. No other deficiencies were noted. These deficiencies did not affect the outcome of the evaluation.

RAB2300:fluazi17.040

Attachment #2

Excerpt from the Cancer Assessment Document prepared by the Cancer Assessment Review Committee (HED) following its evaluation of the carcinogenic potential of fluazinam on January 3, 2001

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1. Combined Chronic Toxicity/Carcinogenicity Study in Sprague-Dawley Rats (1988)

Reference:

Mayfield, R., S. Burton, D. Crook, et al. 1988. Fluazinam technical (B1216): potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks. Huntingdon Research Centre, Ltd., Cambridgeshire, PE18 6ES, England. Report No. ISK 8/87263, August 25, 1988. MRID 42248620. Unpublished.

Mayfield, R., C. Gopinath, and S. Begg. 1999. Addendum to report No. ISK 8/87263. B-1216: potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks. Huntingdon Life Sciences, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Report No. ISK 8/87263 Addendum, March 3, 1999. MRID 44807223. Unpublished.

Lewis, D.L. 2000. Supplement to report no. ISK 8/87263 (MRID#42248620) B-1216: potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks (historical control data). Huntingdon Life Sciences, Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Document No. ISK 8/87263 Supplement, May 31, 2000. MRID 45150201. Unpublished.

A. Experimental Design

In a combined chronic toxicity/carcinogenicity study (MRID 42248620, 44807223, 45150201), B-1216 (Fluazinam technical, 95.3% a.i., lot number 8412-20) was administered to groups of 60 male and 60 female Sprague-Dawley rats at dietary concentrations of 0, 1, 10, 100 or 1000 ppm (0, 0.04, 0.38, 3.82 or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.87 or 53 mg/kg/day for females) for up to 104 weeks. Groups of 10 rats of each sex per dose group were sacrificed at 52 weeks for interim evaluations.

B. Discussion of Tumor Data and Comparison with Historical Control Data

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of fluazinam in the male or female rats [(1)].

A summary of the thyroid gland follicular cell neoplasms seen in the male rats in this study is presented in Table 1. It should be noted that thyroid gland neoplasms described in the study report as "follicular cystadenomas" are included in the table under the category of "follicular adenomas". The incidence of thyroid gland follicular cell adenomas in the male rats in this study was 8%, 9%, 13%, 15% and 17% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. In

(1) Brunsman, L.L. (2000) Fluazinam qualitative risk assessment based on Sprague-Dawley rat and CD-1 mouse dietary studies. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), December 13, 2000. Tox Doc. No. 014401. pp. 4-5.

none of the treated groups was the increased incidence statistically significant by pair-wise comparison with the control group, and there was no statistically significant trend. The incidence of thyroid gland follicular cell adenocarcinomas was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. In neither the 100 ppm group or the 1000 ppm group was the increased incidence statistically significant by pair-wise comparison with the control group, but there was a statistically significant ($p<0.05$) positive trend. The incidence of thyroid gland follicular cell combined adenomas/adenocarcinomas was 8%, 9%, 13%, 18% and 23% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The incidence in the 1000 ppm group was statistically significantly ($p<0.05$) increased by pair-wise comparison with the control group, and there was also a statistically significant ($p<0.05$) positive trend.

In addition, increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats in this study. The incidence of pancreatic islet cell adenomas in the male rats in this study was 8%, 10%, 24%, 14% and 18% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. In none of the treated groups was the increased incidence statistically significant by pair-wise comparison with the control group, and there was no statistically significant positive trend. In addition, the observed incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). For pancreatic islet cell adenomas in male rats, the range of the percent incidence in the historical control data was 0 % to 28% and the mean was 13.8 % [(2)]. This type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Further, no pancreatic islet cell adenocarcinomas were observed in any of the treated male rats in this study. For pancreatic islet cell adenocarcinomas in male rats, the range of the percent incidence in the historical control data was 0 % to 12% and the mean was 4.0 % [(2)]. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in treated males (48% to 71%; controls, 60%) and in treated females (52% to 86%; controls. 68%) and of mammary gland fibroadenomas in treated females (79% to 94%; controls 87%) were also observed in all groups, including controls. These neoplasms are not considered to be treatment-related. A noteworthy increase of tumors was not observed in any other tissues in the treated male or female rats in this study.

Historical control data for thyroid gland follicular cell neoplasms in male Sprague-Dawley rats were provided by the applicant for 20 studies performed at Huntingdon Life Sciences, Huntingdon, England. These studies were started between 1981 and 1990 and had study durations of 101-111 weeks (MRID 45150201). In a separate verbal communication with the applicant on January 9, 2001, EPA was informed that the lesion described in these 20 studies as thyroid gland follicular cell adenomas did not include any lesions described as follicular cell cystadenomas. However, in none of the 20 studies was a lesion described as follicular cell cystadenoma reported. Therefore, the results in this rat study (MRID 42248620) may be directly compared to the results in the 20 historical control studies.

[(2)] Lewis, D.L. (2000). Twenty studies performed at Huntingdon Life Sciences, Huntingdon, England; started between 1981 and 1990; studies of 101-111 weeks duration, MRID 45150201.

Historical Control Data for Thyroid Gland Follicular Cell Neoplasms in Male Sprague-Dawley Rats (Uncensored Data), from MRID 45150201

Follicular cell adenomas	Range	0 - 13%
	Mean	6.5%
Follicular cell adenocarcinomas	Range	0 - 5%
	Mean	1.4%

The study under discussion (MRID 42248620) was initiated in 1985 at Huntingdon Research Centre (England). For thyroid gland follicular cell adenomas in male rats, the range of the percent incidence in the 1981-1990 historical control data (uncensored data) was 0 to 13% and the mean was 6.5%. The percent incidence of thyroid gland follicular cell adenomas in the male rats in this study (censored data) was 8%, 9%, 13%, 15% and 17% for the 0, 1, 10, 100 and 1000 ppm groups, respectively (from Table 1). The incidence at 10 ppm (13%) equaled, and the incidences at 100 ppm (15%) and at 1000 ppm (17%) exceeded the highest percent incidence in the historical control data (13%). For thyroid gland follicular cell adenocarcinomas in male rats, the range of the percent incidence in the 1981-1990 historical control data (uncensored data) was 0 to 5% and the mean was 1.4%. The percent incidence of thyroid gland follicular cell adenocarcinomas in the male rats in this study (censored data) was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively (from Table 1). The incidence at 1000 ppm (6%) exceeded the highest percent incidence in the historical control data (5%).

It is also appropriate to compare the uncensored data for thyroid gland follicular cell neoplasms observed in the male rats in this study to the uncensored historical control data. The percent incidence of thyroid gland follicular cell adenomas in the male rats in this study (uncensored data) was 4/50= 8%, 3/35=9%, 5/40=12%, 5/35=14% and 8/50=16% for the 0, 1, 10, 100 and 1000 ppm groups, respectively (from MRID 42248620). The incidence at 100 ppm (14%) and at 1000 ppm (16%) exceeded the highest percent incidence in the historical control data (13%). The percent incidence of thyroid gland follicular cell adenocarcinomas in the male rats in this study (uncensored data) was 0/50=0%, 0/35=0%, 0/40=0%, 1/35=3% and 3/50=6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively (from MRID 42248620). The incidence at 1000 ppm (6%) exceeded the highest percent incidence in the historical control data (5%).

On January 3, 2001, during its meeting to evaluate the carcinogenic potential of fluazinam, the CARC noted that although there were no statistically significant survival disparities between the control and treated groups of male rats in this study (Cox or Generalized K/W Test), nevertheless the mortality of the control group was 15% higher than that of the male high dose group [(1)]. The CARC requested that a Peto's Prevalence Test analyses of the thyroid gland follicular cell neoplasms observed in the male rats in this study also be performed (in addition to the Exact Trend Test and Fisher's Exact Test already available to the CARC) and considered at the meeting. The results of the Peto's Prevalence Test analyses were made available to the CARC at

Table 1. Fluazinam - 1988 Sprague-Dawley Rat Study - Males

Male Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	10	100	1000
Adenomas [++] (%)	4/48 (8)	3/34 (9)	5/38 (13)	5[a]/34 (15)	8/47 (17)
p =	0.113	0.618	0.353	0.288	0.167
Carcinomas (%)	0/48 (0)	0/34 (0)	0/38 (0)	1/34 (3)	3[b]/47 (6)
p=	0.011*	1.000	1.000	0.415	0.117
Combined (%)	4/48 (8)	3/34 (9)	5/38 (13)	6/34 (18)	11/47 (23)
p =	0.018*	0.618	0.353	0.177	0.041*

from: Brunsman, L.L. (2000) Fluazinam qualitative risk assessment based on Sprague-Dawley rat and CD-1 mouse dietary studies. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), December 13, 2000. Tox Doc. No. 014401. p. 6.

[+]Censored Data. Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

[++] Tumors described in the study report as follicular cystadenomas are included in this table under the category of follicular adenomas.

[a]First adenoma observed at week 70, dose 100 ppm.
[b]First carcinoma observed at week 68, dose 1000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid gland follicular cell tumors in any interim sacrifice animals.

Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

the meeting and were subsequently presented in a memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED) dated January 4, 2001 [3]. The results of the Peto's Prevalence Test analyses are presented in Table 2. For follicular cell adenocarcinomas in males, results of the Peto's Prevalence Test showed a statistically significant positive trend and a borderline statistically significant ($p= 0.056$) increase by pair-wise comparison of the 1000 ppm male group with the controls (7% vs 0% in controls), indicating the increased incidence of thyroid tumors had a malignant component to it. For combined follicular cell adenomas/adenocarcinomas, Peto's Prevalence Test also showed a statistically significant increase by pair-wise comparison of the high dose male group with the controls (26% vs 9% in controls)

C. Non-neoplastic Lesions

A suggestion of possibly increased goitrogenic activity was observed in this study. At the highest dose level tested (1000 ppm), absolute and relative thyroid weights were increased ($p<0.01$) in males at 52 weeks (but not at 104 weeks). Absolute mean thyroid weights at 52 weeks were 29.6, 31.7, 27.2, 31.0 and 42.4 grams for the 0, 1, 10, 100 and 1000 ppm dose groups respectively. Relative mean thyroid weights were 3.91 and 6.40 for the 0 and 1000 ppm dose groups, respectively. An increased incidence of enlarged thyroids ($p<0.05$) was also observed during gross examination of the males at 104 weeks. The incidences were 2/50, 2/50, 4/50, 4/50 and 9/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively. In addition, a slightly increased incidence (not statistically significant) of thyroid follicular cell hyperplasia was observed at 104 weeks in males; 1/50, 2/35, 1/40, 0/35 and 4/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively. A slightly increased incidence (not statistically significant) of thyroid follicular cell hyperplasia was also observed at 104 weeks in females; 1/50, 0/25, 0/27, 0/32 and 5/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively.

At the LOAEL in this study (100 ppm), treatment-related histopathological lesions were observed in the liver of males (centrilobular sinusoidal dilatation and pericholangitis) and in the liver of females (centrilobular sinusoidal dilatation, pericholangitis and eosinophilic hepatocytes). In addition, in males, a statistically significant ($p<0.01$) increased incidence of atrophy of the testes was observed (controls, 7/50; 100 ppm, 19/49) and in females a statistically significant ($p<0.01$) increased incidence of exocrine atrophy of the pancreas was observed (controls, 3/50; 100 ppm, 13/50).

At the highest dose tested in this study (1000 ppm), statistically significant ($p<0.01$) increased incidences of several histopathological lesions in the livers of both male and female rats were observed. These lesions included centrilobular sinusoidal dilatation, pericholangitis, eosinophilic hepatocytes, centrilobular hepatocyte rarefaction and vacuolation, and centrilobular fat.

[3] Brunsman, L.L. (2001) Fluazinam male rat thyroid follicular cell Peto's prevalence analyses based on Sprague-Dawley rat dietary study. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), January 4, 2001. Tox. Doc. No. 014428. p. 3.

Table 2. Fluazinam - 1988 Sprague-Dawley Rat Study - Males

Male Thyroid Gland Follicular Cell Tumor Rates[+]
and Peto's Prevalence Test Results (p values)

	Dose (ppm)				
	0	1	10	100	1000
Adenomas [++] (%)	4/46 (9)	3/33 (9)	5/37 (14)	5[a]/33 (15)	8/41 (20)
p =	0.283	0.144	0.095	0.150	0.079
Carcinomas (%)	0/47 (0)	0/33 (0)	0/37 (0)	1/33 (3)	3[b]/43 (7)
p=	0.038*	-	-	0.079	0.056
Combined (%)	4/47 (9)	3/33 (9)	5/37 (14)	6/33 (18)	11/43 (26)
p =	0.100	0.139	0.093	0.068	0.022*

from: Brunsman, L.L. (2001) Fluazinam male rat thyroid follicular cell Peto's prevalence analyses based on Sprague-Dawley rat dietary study. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), January 4, 2001. Tox. Doc. No. 014428. p. 3.

[+]Censored Data. Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before observation of the first tumor.

[++] Tumors described in the study report as follicular cystadenomas are included in this table under the category of follicular adenomas.

[a]First adenoma observed at week 70, dose 100 ppm.
[b]First carcinoma observed at week 68, dose 1000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid gland follicular cell tumors in any interim sacrifice animals.

Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Statistically significant ($p<0.05$) increased incidences of bile duct hyperplasia were also observed in both males and females and centrilobular hepatocyte necrosis in females. In addition, in males, statistically significant increases were observed in the following histopathological lesions: pneumonitis and alveolar epithelialization in lungs, exocrine atrophy in pancreas, cortical tubular basophilia in kidney, and atrophy of testes. In females, statistically significant increases were observed in the following histopathological lesions: alveolar epithelialization in lungs; and exocrine atrophy, acinar epithelial vacuolation and acinar epithelial fat in pancreas.

Treatment-related increases in the incidence and/or severity of vacuolation of the white matter in the brain or spinal cord were not observed in either the male or female rats in this study.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CARC considered the dosing in this study to be adequate but not excessive for assessment of carcinogenic potential. This determination was based on decreased body weight gain observed at the 1000 ppm dose level and the histopathological lesions observed at the 100 ppm and 1000 ppm dose levels. Males receiving the 1000 ppm diet weighed 6–16% ($p<0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed ≤8% less food than controls at each weekly interval. Females receiving the 1000 ppm diet weighed 7–24% ($p<0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed ≤18% less food than controls at each weekly interval. The food utilization factor or the food efficiency suggested that the reduced body weight gain was due in part to toxicity of the test material.

Treatment-related findings indicated that the test material was toxic to the following organs: liver, pancreas, lungs, and possibly thyroid gland in both sexes, and kidneys and testes in males. The primary target organ appeared to be the liver. Liver toxicity was manifested by increased organ weight and increased incidences of gross and microscopic lesions. Absolute liver weights were increased by at least 7-27% and relative weights by 17-63% in both sexes at 1000 ppm. Gross lesions in the liver consisted of pale, swollen or accentuated markings on the livers at 1000 ppm in males and enlarged, pitted, or mottled livers at 1000 ppm in females. Treatment-related microscopic liver lesions at 100 ppm and at 1000 ppm were described previously under "Non-neoplastic Lesions". Centrilobular hepatocyte vacuolation and centrilobular fat were also seen in 1000 ppm group male and female rats at interim sacrifice.

Histopathological findings observed at 1000 ppm in the pancreas, lungs and thyroid gland in males and females, and in the kidney and testes of males were described previously under "Non-neoplastic Lesions".

Histopathologic assessment of the brain and spinal cord of rats in the control and 1000 ppm dose groups showed no treatment-related effect on vacuolation of white matter.