B

DATA EVALUATION REPORT

014613

FLUAZINAM

STUDY TYPE: CHRONIC ORAL TOXICITY FEEDING – RAT [OPPTS 870.4100 (§83-1a)]
MRID 44839901, 44807213

Prepared for

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Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Chronic oral toxicity feeding - Rat; [OPPTS 870.4100 (§83-1a)]

DP BARCODE: D258235
P.C. CODE: 129098

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

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

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

CITATION: Chambers, P., Brennan, C., Crook, D., et al. (1993) B-1216 Toxicity to rats by dietary administration for 2 years. Huntingdon Research Centre Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England. Document No. ISK 43/920649, June 14, 1993. MRID 44839901. Unpublished.

Broadmeadow, A. (1983). B-1216: Four-week toxicity study in dietary administration to CD rats. Life Science Research, Stock, Essex, CM4 9PE, England. Document No. 82/ISK035/544. May 23, 1983. MRID 44807213. Unpublished.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 chronic oral toxicity study (MRID 44839901), technical grade fluazinam (95.3% a.i., Batch # 8412-20) was administered to 25 Crl:CD®(SD)BR rats/sex/dose in the diet at dose levels of 0, 25, 50, or 100 ppm for 104 weeks (0, 1.0, 1.9, or 3.9 mg/kg/day for males; 0, 1.2, 2.4, or 4.9 mg/kg/day for females). A four-week range-finding study in rats was also conducted using 0, 50, 250, or 3000 ppm (MRID 44807213).

No clinical signs of toxicity were observed, and survival rates were unaffected by treatment. At study termination, survival rates for the 0, 25, 50, or 100 ppm groups were 32, 52, 28, and 36% for the males, respectively, and 72, 56, 72, and 52% for the females, respectively. No treatment-related effects on mean absolute body weights, body weight gain, food consumption, or water consumption were noted.

No treatment-related differences were observed in hematology analysis or urinalysis, and treatment-related clinical chemistry changes were limited to a transient increase in total serum cholesterol in high-dose females at Week 52 (154% of controls, $p<0.01$). Relative liver weights were increased in high-dose females at study termination (124% of controls; $p<0.01$), but were not accompanied by any histopathological correlates. The increased relative liver weights and transient increase in cholesterol were therefore not considered adverse.

Treatment with fluazinam appeared to affect the testes. High-dose decedent males (those not surviving to study termination) had an increased incidence of small and/or flaccid testes (11/16 or 69%, 8/16 or 50%, respectively;) as compared with decedent controls (4/17 or 24% for both) during macroscopic examination. Microscopic examination revealed a corresponding increased incidence of marked tubular atrophy in the testes of these 100 ppm decedent males (8/16; 50%) as compared with decedent controls (3/17; 18%); however, this increase was not statistically significant and did not show a dose-response when considering the average severity ranking for the decedent males (2.5, 3.4, 2.6, and 3.4 for the 0, 25, 50, and 100 ppm decedent males, respectively). When considering all 100 ppm males (decedent + terminal), macroscopic examination did not reveal an increased incidence of small and/or flaccid testes (16/25 vs. 16/25 controls), but the overall severity of testicular tubular atrophy was increased in the high-dose males (3.1) as compared with controls (2.6). Because testicular atrophy was also an effect noted in male rats following dietary treatment with 100 or 1000 ppm in another chronic toxicity/carcinogenicity study (MRID 42248620), it is considered an effect of treatment in this study.

At the doses tested, there was not a treatment-related increase in tumor incidences when compared to controls.

A LOAEL of 100 ppm (3.9 mg/kg/day) was identified for male rats based on increased testicular atrophy, with a corresponding NOAEL of 50 ppm. (1.9 mg/kg/day). A LOAEL could not be identified for females. The NOAEL for females was therefore ≥ 100 ppm (4.9 mg/kg/day).

This chronic oral toxicity study in the rat is classified as **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a chronic oral toxicity study [OPPTS 870. 4100 (§83-1a)] in rats.

Range-Finding Study

In a 4-week oral range-finding study (MRID 44807213), technical grade fluazinam (96.3% a.i., Batch # 8203) was administered to 10 CD (Sprague-Dawley) rats/sex/dose in the diet at dose levels of 0, 10, 50, 250, or 3000 ppm for 4 weeks (0, 1.0, 5.1, 26.4, or 302 mg/kg/day for males; 0, 1.1, 5.3, 25.9, or 309 mg/kg/day for females).

The following treatment-related effects were observed at 3000 ppm in both males and females: decreased body weight gain, decreased food consumption, increased serum phospholipid, increased total cholesterol, increased absolute liver weights, and increased relative liver/body weight ratios. In addition, increased incidences of histopathologic findings were observed in the

liver of males (increased periacinar hypertrophy) and in the liver of females (increased single cell necrosis). The following treatment-related effects were also observed at 250 ppm: decreased body weight gain (females), decreased food consumption (females), increased serum phospholipid (females), increased total cholesterol (males and females), increased relative liver/body weight ratios (females), and increased histopathology in the liver of males (increased periacinar hypertrophy). In an addendum to this study, there was no effect of treatment on the incidence or degree of white matter vacuolation in the brain of male or female rats of the high dose group (3000 ppm), compared with controls.

The LOAEL was 250 ppm (26.4 mg/kg/day for males; 25.9 mg/kg/day for females), based on decreased body weight gain (females), decreased food consumption (females), increased serum phospholipid (females), increased total cholesterol (males and females), increased relative liver/body weight ratios (females), and increased histopathology in the liver of males (increased periacinar hypertrophy). The NOAEL was 50 ppm (5.1 mg/kg/day for males; 5.3 mg/kg/day for females).

This range-finding study is classified as **Acceptable/Non-Guideline**. It does **not** satisfy the Subdivision F guideline requirement for a subchronic oral toxicity study [OPPTS 870.3100 (82-1)] in rats.

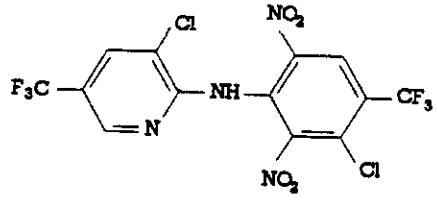
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: B-1216 Technical (Fluazinam technical)

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



2. Vehicle and/or positive control
   The test chemical was administered in the feed, powdered Labsure LAD No. 2.

3. Test animals

Species: rat
Strain: Crl:CD®(SD)BR
Age and weight at study initiation: approximately 5-6 weeks old, males: 158-194 g, females: 118-145g
Source: Charles River, Portage, Michigan, U.S.A.
Housing: housed in suspended cages with wire mesh floors, 5 rats/sex/cage during the treatment period
Diet: powdered Labsure LAD No. 2 diet was available *ad libitum*
Water: tap water was available *ad libitum*
Environmental conditions:
Temperature: 21 ± 2°C
Humidity: 50 ± 10%
Air changes: not stated
Photoperiod: 12 hr dark/12 hr light
Acclimation period: approximately 2 weeks

B. STUDY DESIGN

1. In life dates - start: November 13, 1989; end: November 15, 1991

2. Animal assignment

Groups of 25 animals were randomly assigned to the test groups in Table 1 based on body weights.

TABLE 1: Study design

Test group	Dose (ppm)	Dose (mg/kg/day)	
		Male	Female
Control	0	0	0
Low	25	1.0	1.2
Mid	50	1.9	2.4
High	100	3.9	4.9

Data taken from p. 27 and text table on p. 28, MRID 44839901.

The test material was administered to the animals in the feed for 104 weeks.

3. Dose selection rationale

Dose selection was by the Sponsor with reference to available toxicity data and on the basis of a previous long-term carcinogenicity/toxicity study on B-1216 (MRID 42248620). In that study (MRID 42248620), male and female rats were fed diets containing 1, 10, 100, or 1000 ppm B-1216. Treatment with 100 or 1000 ppm B-1216 resulted in changes in body weight, food intake, efficiency of food utilization, a slight anemia, elevated cholesterol, increased liver weight, an increased number of macroscopic liver and testicular lesions, and a microscopically observed increase in lung, liver, pancreas, lymph node, and testis lesions. The treatment levels 1 or 10 ppm were considered no observable effect levels.

In a 4-week toxicity study in which male and female rats were fed diets containing 0, 10, 50, 250, or 3000 ppm fluazinam, a LOAEL of 250 ppm was identified based on decreased body weight gain (females), decreased food consumption (females), and hepatic changes including increased serum phospholipids (females) and total cholesterol (males and females), an increased liver/body weight ratio (females), and increased incidence of periacinar hypertrophy (males) (MRID 44807213; see Appendix).

4. Diet preparation and analysis

A pre-mix was prepared each week by grinding the test substance directly into the diet and mixing in an inflated polythene bag for a minimum period of 3 minutes. The required concentrations were then prepared by direct dilution of the pre-mix with further quantities of untreated diet; homogeneity being achieved by mixing in a double cone blender for a minimum period of 7 minutes. Treated diets were prepared weekly and were stored at ambient temperature in the animal room.

The study author stated that before the start of treatment, the diet mixing procedures were checked by chemical analysis of trial diet mixes of the low- and high-dose levels to confirm that the proposed procedures produced homogenous diets, that the accuracy of mixing was acceptable, and that the concentration of test material in the diet remain unchanged between preparation and the end of administration. In Addendum 4 (MRID 44839901. p. 1165), it is stated that the homogeneity and stability of rodent diet formulation containing B-1216 at concentrations of 1 and 1000 ppm was undertaken during an earlier study, and the results are reported in HRC Report No. ISK 8/87263, Addendum 3 (MRID 42248620).

The accuracy of the diet preparation was assessed by analyzing samples prepared during weeks 1, 13, 26, 39, 52, 65, 78, 91, and 104.

Results

Homogeneity analysis: Although it was stated by the study author that the homogeneity of the low- and high-dose levels was tested, the actual homogeneity data were not provided in this study report. The reviewer was referred to a previous study, MRID 42248620. The results of the homogeneity analysis in MRID 42248620 were:

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 ppm. **1000 ppm**: The concentrations ranged from 978 to 1010 ppm, with a standard deviation of 1.18 ppm.

Stability analysis: Although it was stated by the study author that the stability of the low- and high-dose levels was tested, the actual stability data were not provided in this study report. The reviewer was referred to a previous study, MRID 42248620. The results of the stability analysis in MRID 42248620 were:

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 the test diet samples analyzed during the study were found to be acceptable. The range of mean concentrations as a percentage of nominal concentrations were 98-103%, 91-101%, and 94-106% for the 25, 50, or 100 ppm test diets, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. Statistics

Data were analyzed separately by sex. Data pertaining to food and water consumption were analyzed on a cage basis. For all other parameters, the analyses were carried out using the individual animals as the basic experimental unit.

To analyze data for food or water consumption, body weight, organ weight, or clinical pathology, the following sequence of statistical tests were used: 1) if the data consisted predominately of one particular value (relative frequency of the mode exceed 75%), the proportion of animals with values different from the mode was analyzed by Fisher's exact test or Mantel's test; 2) Bartlett's test was applied for heterogeneity of variances between treatments; if variances were unequal, a logarithmic transformation was tried; 3) a one-way analysis of variance was carried

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out on untransformed or transformed data. The Kruskal-Wallis analysis of ranks was used on data where heterogeneity of variance was still present; 4) analysis of variance was followed by Student's t test and Williams' test for a dose-related response; Kruskal-Wallis analyses were followed by non-parametric equivalents. Analysis of covariance was applied in place of analysis of variance for organ weight data, with the final body weight used as a covariate.

C. METHODS

1. Observations

Animals were observed at least once daily for any signs of behavioral changes, reaction to treatment, or ill health. Further checks were made early in each working day and again in the afternoon to look for dead or moribund animals. A detailed palpation of each rat was performed once weekly in order to record details of all new palpable masses. After the initial observation of these masses, the progression or regression of each mass was followed by recording the dimensions of each mass every two weeks.

2. Body weight

Individual body weights were recorded at the time of allocation of animals to groups, on the day of commencement of treatment, and once a week thereafter.

3. Food consumption and compound intake

The quantity of food consumed by each cage of rats was recorded weekly. Food intake per rat (g/rat/week) was calculated using the amount of food given to and left by each group and the number of rats surviving in each cage. Food utilization (g food consumed/g body weight gain) was calculated during the first 26 weeks from body weight and food consumption data as weight of food consumed per unit gain in body weight. The group mean intake of material (mg/kg/day) was calculated at weekly intervals.

4. Water consumption

Water consumption was measured by weight over daily periods during Weeks 12, 25, 51, 77, and 103 for all cages. Daily monitoring by visual appraisal of the water bottles was maintained throughout the study.

5. Ophthalmoscopic examination

Eyes were examined in all animals before study initiation and on each animal in the control and high-dose level during weeks 13, 52, and 103 by means of a Keeler indirect ophthalmoscope.

6. Blood was collected from the orbital sinus from 10 male and 10 female fasted rats from each group for hematology and clinical chemistry analyses during Weeks 13, 26, 52, and 78 and at termination. The rats were fasted (food) overnight prior to all sample collections at each scheduled interval. Where possible, the same rats were used for a particular investigation. The animals were fasted overnight and anesthetized before sampling. The CHECKED (X) parameters were examined.

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*		Reticulocyte count
	Blood clotting measurements*		Nucleated red blood cell count
X	(Thromboplastin time)	X	Cell morphology
	(Clotting time)		
	(Prothrombin time)		

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

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*
		X	Total bilirubin*
	ENZYMES	X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		
X	Creatine phosphokinase*		
	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

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

6. Urinalysis

Overnight urine samples were collected from 10 male and 10 females rats in each group during Weeks 13, 26, 52, and 78 and at termination. The rats were fasted (food and water) overnight. The CHECKED (X) parameters were examined.

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

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

7. Sacrifice and pathology

All animals were killed by carbon dioxide asphyxiation on completion of 104 weeks of treatment and were subjected to gross pathological examination. The CHECKED (X) tissues from all animals were collected for histological examination. All tissues (excluding the Harderian gland, Zymbal's gland, larynx and pharynx, mammary gland, tongue, and vagina) collected from control and high-dose animals sacrificed at termination and from all animals dying or killed *in extremis* were examined microscopically. Additionally, the lungs, liver, and kidneys from all rats in the low- and mid- dose groups, the testes and epidydimides from low- and mid-dose males, the spleen and pancreas from low- and mid-dose females, and any macroscopically abnormal tissue in any animal were examined microscopically. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT	X	NEUROLOGIC
x	Tongue	x	Aorta*	xx	Brain*
x	Salivary glands*	x	Heart*	x	Periph. nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*	x	Eyes (optic n.)
x	Jejunum*	x	Thymus*		
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*+ (with	x	Parathyroids*
x	Pancreas*		Epididymides)	x	Thyroids*
		x	Prostate	x	Harderian gland
	RESPIRATORY	x	Seminal vesicle		Zymbal's gland
x	Trachea	x	Ovaries		
x	Lung*	x	Uterus*		**OTHER**
x	Nose	x	Vagina	x	Bone
x	Pharynx			x	Skeletal muscle
x	Larynx			x	Skin
				x	All gross lesions and masses*
				x	Middle ear

* Required for carcinogenicity studies based on Subdivision F Guidelines.
+ Organ weight required in carcinogenicity studies.

II. RESULTS

A. OBSERVATIONS

1. Clinical Signs of Toxicity

No treatment-related clinical signs were observed and no significant increase in the incidence of palpable tissue masses were observed in treated males or females.

2. Mortality

Survival rates were not effected by treatment in either sex. At study termination, the survival rates for the 0, 25, 50, or 100 ppm groups were 32, 52, 28, and 36% for the males, respectively, and 72, 56, 72, and 52% for the females, respectively.

B. BODY WEIGHT

Although the study authors reported the group mean body weights, the standard deviations of the group means and the statistical analysis of the body weight data were not provided in the study report. The reviewer calculated values for selected weeks (see Table 2), and found no statistically significant differences as analyzed using ANOVA. Mean body weight gains were significantly different only in high-dose females during

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weeks 75-104 (-14g vs. 60 g for controls, $p<0.05$) and for weeks 0-104 (356 g vs. 462 g for controls, 77%, $p<0.05$)

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

Week	Concentration (mg/kg/day)							
	0	25	50	100	0	25	50	100
	Males				Females			
	Mean body weights (g) [a]							
0	175 ± 7.3	177 ± 8.0	174 ± 7.9	174 ± 8.0	133 ± 8.5	132 ± 6.0	133 ± 4.8	132 ± 5.4
28	683 ± 85.1	652 ± 64.6	648 ± 84.4	650 ± 78.0	337 ± 31.0	328 ± 32.9	332 ± 26.6	346 ± 46.9
52	797 ± 109.8	754 ± 88.6	763 ± 116.7	759 ± 127.3	421 ± 52.7	421 ± 58.6	430 ± 52.5	441 ± 77.8
75	888 ± 154.7	844 ± 125.1	816 ± 84.6	808 ± 123.2	534 ± 68.0	510 ± 89.5	540 ± 62.1	521 ± 91.0
104	746 ± 112.0	776 ± 143.0	815 ± 122.5	730 ± 104.5	594 ± 101.2	549 ± 161.1	583 ± 116.6	489 ± 120.0
	Body weight gain (g)							
0-28 [b]	508 ± 80.7	476 ± 61.9	474 ± 81.8	476 ± 74.2	204 ± 26.8	196 ± 30.7	199 ± 25.7	215 ± 44.6
28-52 [b]	114 ± 38.5	101 ± 59.1	114 ± 42.0	109 ± 65.7	85 ± 32.6	93 ± 31.1	98 ± 35.5	94 ± 37.9
52–75 [b]	88 ± 92.1	88 ± 52.9	78 ± 76.3	67 ± 52.7	114 ± 32.8	93 ± 53.7	111 ± 32.0	91 ± 46.3
75–104	-71 ± 85.4	-63 ± 91.6	10 ± 88.0	-14 ± 52.2	60 ± 82.6	36 ± 110..9	54 ± 75.8	-14* ± 76.9
0–75	712 ± 151.9	668 ± 123.2	642 ± 84.9	634 ± 120.8	401 ± 64.5	378 ± 89.0	406 ± 62.1	390 ± 89.6
0–104	573 ± 106.0	602 ± 138.7	643 ± 118.9	560 ± 102.5	462 ± 99.9	417 ± 158.6	450 ± 117.8	356* ± 119.1

Data taken from Text Table on p. 27, and Table 2 and Appendix 1, pp. 40-44, 171-226, MRID 44839901.

[a] Standard deviation calculated by reviewer; no statistically significant differences observed as analyzed by ANOVA.

[b] Means and standard deviations calculated by the reviewer; no statistically significant differences as analyzed by ANOVA.

Significantly different from control: *$p < 0.05$ by William's Test.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Again, mean food consumption values were provided, but the standard deviations of the group means and the statistical analysis of the food consumption data were not provided in the study report. There do not appear to be substantial differences in food consumption, and statistical analysis of the overall cumulative group mean food consumption (g/rat) did not reveal any statistically significant differences. Overall mean for consumption for the 0, 25, 50, and 100 ppm groups was 18,811, 18,552,

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18,089, and 18,577 g/rat for the males, respectively, and 13,872, 13,751, 13,850, and 13,940 g/rat for females, respectively.

2. Water consumption

No statistically significant differences in cumulative water intake were observed in treated males or females as compared with controls.

3. Compound consumption

Compound consumption data are presented in Table 1. Males received an average of 0, 1.0, 1.9, or 3.9 mg/kg body weight, while females received 0, 1.2, 2.4, or 4.9 mg/kg body weight.

4. Food utilization

No treatment-related differences in food utilization for males or females were noted during the first 26 weeks of treatment. Food utilization (g food consumption/g bw gain) for the 0, 25, 50, and 100 ppm groups was 9.9, 10.3, 10.2, or 10.3 for males, respectively, and 16.2, 16.9, 16.9, or 16.3 for females, respectively.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related effects of B-1216 on the eyes were noted in males or females.

E. BLOOD WORK

1. Hematology

Statistically significant differences in parameters observed during the study either were not biologically significant, or did not show a dose- or time-response.

2. Clinical chemistry

High-dose females had increased cholesterol levels at 52 weeks (+154%; $p < 0.01$) and 78 weeks (+168%; not significant) as compared with controls, but had cholesterol levels comparable to controls at 104 weeks. In general, other statistically significant differences in clinical chemistry parameters in treated males and females (including: decreased albumin in all treated males at Weeks 13 and 26; increased glutamic-oxaloacetic transaminase in all treated males at Week 13 and in mid-and high-dose males at Week 52; increased glucose in high-dose females at Weeks 13, 26, and 52; decreased creatinine phosphokinase in mid- and high-dose females at week 52; increased creatinine in all treated females at week 26; and occasional changes in electrolytes in treated males and females) were not biologically significant, were transient, and/or were not related to dose.

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F. URINALYSIS

Statistically significant differences in protein and pH observed in treated males and females were not considered an effect of treatment because the changes were not biologically significant and/or were not related to dose.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

At the terminal kill, high-dose males had increased testes and epididymides weights relative to body weight (5.12 g vs. 4.38 for controls; 117% of controls; $p<0.01$), and high-dose females had increased liver weight relative to body weight (23.9 vs. 19.3 for controls; 124% of controls; $p< 0.01$). No other statistically significant differences were noted.

2. Gross pathology

Macroscopic evaluation of rats found dead or killed in extremis revealed a greater number of decedent 100 ppm males with small and/or flaccid testes (11/16 and 8/16 decedents, respectively) compared with controls (4/17 control decedents for both). This effect was not observed in the 100 ppm group surviving to the terminal kill at week 105. No other potential treatment-related gross macroscopic changes were observed.

3. Microscopic pathology

a. Non-neoplastic

The authors reported an increased incidence of marked tubular atrophy in the testes of 100 ppm decedent males (8/16; 50%) as compared with decedent controls (3/17; 18%). This increase was not statistically significant and did not show a dose-response when considering the average severity ranking for the decedent males (see Table 3). The incidence and severity of testicular tubular atrophy were not increased when comparing treated males surviving to termination with the control group (Table 3). Although the overall incidence of testicular tubular atrophy was not increased in all treated males (decedent + terminal rats), the severity of the lesion was increased in high-dose rats (3.1) as compared with controls (2.6).

Additional non-neoplastic histopathologic findings for male and female rats in this study are presented in Tables 4 and 5, respectively. The study authors reported that examination of the lungs of decedent and terminal animals combined revealed adenomatous hyperplasia, also known as alveolar adenomatosis (2 males and 1 female from the mid-dose group and 1 female in the high-dose group) and alveolar

epithelialization (1 male from the low-dose group, 2 females from the mid-dose group, and 1 male and 3 females from the high-dose group) in treated males and females, while evaluation of the pancreas revealed an increased incidence of exocrine acinar atrophy in mid- and high-dose females (14/25 and 12/25, respectively) as compared with controls (8/25). A previous study recorded similar treatment-related findings (ISK 8/87263; MRID No. 42248620), but these lesions did not attain statistical significance and/or were not related to dose in this study (MRID 44839901).

Other non-neoplastic findings discussed by the study authors included eosinophilic hepatocytes in all groups of males and females, moderate hemosiderosis in the spleen of mid- and high-dose females, and minimal extramedullary hemopoiesis in terminal high-dose male rats. The findings did not attain statistical significance and/or were not related to dose and were therefore not considered to be an effect of treatment.

Additional findings were included in Tables 4 and 5 because they were considered to be treatment-related effects in a previously conducted chronic feedng/ carcinogenicity study in rats (ISK 8/87263; MRID No. 42248620) at dose levels of ≥100 mg/kg/day. They are included in Tables 4 and 5 for the purpose of comparison and to assist in the determination of a NOAEL for these effects when both studies are considered together.

TABLE 3: Tubular atrophy in the testes of male rats fed B-1216 in the diet for up to 104 weeks

Tubular atrophy	Dose (mg/kg/day)											
	0			25			50			100		
	Dead[a]	Terminal	Total	Dead	Terminal	Total	Dead	Terminal	Total	Dead	Terminal	Total
No. examined	17	8	25	12	13	25	18	7	25	16	9	25
Trace	2	2	4	0	6	6	2	1	3	1	1	2
Minimal	4	1	5	1	1	2	2	1	3	1	2	3
Moderate	1	0	1	2	1	3	1	0	1	2	0	2
Marked	3	3	6	4	1	5	3	1	4	8	1	9
Total	10	6	16	7	9	16	8	3	11	12	4	16
Average severity ranking [b]	2.5	2.7	2.6	3.4	1.7	2.4	2.6	2.3	2.5	3.4	2.3	3.1

Data taken from Table 12b, p. 113, MRID 44839901.

[a] Dead. = decedents: the animals that were killed *in extremis* or found dead; Terminal = those animals that survived to the scheduled termination date of 104 weeks; Total = decedents and terminal animal data combined

[b] The average severity rankings as calculated by reviewer based on the assigned severity ratings of the lesion: 1 = trace, 2 = minimal, 3 = moderate, 4 = marked (Table 12b, p. 113, MRID 44839901).

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Table 4. Selected non-neoplastic microscopic findings in male rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)			
	0	25	50	100
Lungs, No. animals examined	25	25	25	25
Alveolar adenomatosis	0	0	2	0
Pneumonitis	4	5	1	6
Alveolar epithelialization	0	1	0	1
Aggregates of alveolar macrophages	8	7	6	4
Liver, No. animals examined	25	25	25	25
Eosinophilic hepatocytes	11	17	11	15
Centrilobular hepatocyte vacuolation	7	4	7	9
Centrilobular hepatocyte necrosis	0	0	0	1
Bile duct hyperplasia	16	17	11	13
Pancreas, No. animals examined	25	25	25	25
Acinar epithelial vacuolation	1	1	1	0
Exocrine acinar atrophy	12	4	11	9
Thyroid gland, No. animals examined	24	12	18	25
Follicular hyperplasia	0	0	0	2
Spleen, No. animals examined	25	17	20	25
Hemosiderosis	11	11	14	14
Extramedullary hemopoiesis	9	12	12	13

Data taken from Table 12b (pp. 92-134), MRID 44839901.

Table 5 Selected non-neoplastic microscopic findings in female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)			
	0	25	50	100
Lungs, No. animals examined	25	25	25	25
Alveolar adenomatosis	0	0	1	1
Pneumonitis	2	4	6	3
Alveolar epithelialization	0	0	2	3
Aggregates of alveolar macrophages	2	4	4	6
Liver, No. animals examined	25	25	25	25
Eosinophilic hepatocytes	9	13	16	15
Centrilobular hepatocyte vacuolation	3	10	12	4
Centrilobular hepatocyte necrosis	0	1	1	3
Bile duct hyperplasia	8	7	8	9
Pancreas, No. animals examined	25	25	25	25
Acinar epithelial vacuolation	0	1	0	4
Exocrine acinar atrophy	8	7	14	12
Thyroid gland, No. animals examined	25	13	7	25
Follicular hyperplasia	0	0	0	2
Spleen, No. animals examined	25	25	25	25
Hemosiderosis	20	19	23	23
Extramedullary hemopoiesis	19	16	19	19

Data taken from Table 12b (pp. 135-169), MRID 44839901.

Microscopic evaluation of animals killed *in extremis* or those that died prior to the scheduled termination date did not reveal any consistent cause of death related to treatment.

b. Neoplastic

Selected neoplastic microscopic findings in male and female rats are presented in Table 6. No treatment-related neoplastic lesions were observed. An increased incidence of pituitary adenocarcinomas in female rats at 100 ppm (5/25 vs 1/24 for controls) was considered to be of no concern because an increase in this same type of tumor was not observed in a previously conducted chronic feeding/ carcinogenicity study in rats at doses up to 1000 ppm (ISK 8/87263; MRID No. 42248620). Similarly, an increased incidence of mammary gland fibroadenomas in female rats at 100 ppm (15/20 vs 8/15 for controls) and of mammary gland adenocarcinomas in female rats at 100 ppm (7/20 vs 2/15 for controls) were also considered to be of no concern because increases in these same types of tumors again were not observed in the previously conducted chronic feeding/ carcinogenicity study in rats at doses up to 1000 ppm.

Additional findings were included in Table 6 (in pancreas, thyroid gland and adrenal gland) because slight increases in these same tumor types were observed in the previously conducted chronic feedng/carcinogenicity study in rats (ISK 8/87263; MRID No. 42248620). They are included in Table 6 for the purpose of comparison.

Table 6 Selected neoplastic microscopic findings in male and female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)			
	0	25	50	100
Males				
Pituitary gland, No. animals examined	25	18	20	25
Pituitary adenoma	10	10	7	9
Pituitary adenocarcinoma	0	0	0	1
Combined	10	10	7	10
Pancreas, No. animals examined	25	17	20	25
Islet cell adenoma	4	7	2	5
Islet cell carcinoma	2	0	0	0
Combined	6	7	2	5
Thyroid gland, No. animals examined	24	12	18	25
Follicular adenoma	3	1	0	2
Follicular carcinoma	0	1	0	1
Combined	3	2	0	3
Mammary gland, No. animals examined	4	3	1	2
Fibroadenoma	1	0	0	0
Adenocarcinoma	0	1	0	0
Adrenal gland, No. animals examined	25	22	23	25
Pheochromocytoma (benign)	5	6	6	2

Table 6 Selected neoplastic microscopic findings in male and female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)			
	0	25	50	100
Females				
Pituitary gland, No. animals examined	24	21	18	25
Pituitary adenoma	11	16	16	11
Pituitary adenocarcinoma	1	1	0	5
Combined	12	17	16	16
Pancreas, No. animals examined	25	25	25	25
Islet cell adenoma	0	1	1	1
Islet cell carcinoma	0	0	1	1
Combined	0	1	2	2
Thyroid gland, No. animals examined	25	13	7	25
Follicular adenoma	0	0	0	0
Follicular carcinoma	0	0	0	1
Combined	0	0	0	1
Mammary gland, No. animals examined	15	20	19	20
Fibroadenoma	8	6	6	12
Fibroadenoma with epithelial atypia	0	1	4	3
Combined	8	7	10	15
Adenocarcinoma	2	3	4	7
Adrenal gland, No. animals examined	25	25	25	25
Pheochromocytoma (benign)	0	0	0	1

Data taken from Table 12a (pp. 86-91), MRID 44839901.

III. DISCUSSION

A. DISCUSSION

No treatment-related clinical signs of toxicity were observed, and survival rates were unaffected by treatment. Body weights and body weights gains were also generally unaffected by treatment, with the only statistically significant differences observed in high-dose females during weeks 75-104 and weeks 0-104. The changes in body weight gains were not accompanied by differences in food consumption or food utilization. Because the changes in body weight gain occurred toward the end of the study, the reviewer agrees with the study author that this change was not an effect of treatment, but rather the result of natural variation in aging animals influencing the mean body weight. No differences in food consumption or food utilization were observed in any other treated groups as compared with controls, and no differences in water consumption were observed. Ophthalmoscopic examination did not reveal any treatment-related abnormalities.

No treatment-related changes in hematology or urinalysis were observed, and effects on serum biochemistry were confined to increased serum cholesterol in high-dose females at week 52 ($p<0.01$) and possibly week 78 (not significant). Increased cholesterol levels were also observed in a 4-week dietary toxicity study (MRID 44807213; see Appendix)

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in male and female rats fed 250 ppm or 3000 ppm B-1216, and in male and female rats fed 1000 ppm B-1216 for up to 2 years (MRID 42248620). Although treatment-related, the transient nature of the increase along with the lack of any histological liver changes, however, suggest that this increased elevation of cholesterol levels in the high-dose females in this study was not toxicologically significant.

Treatment with B-1216 appeared to effect the testes. High-dose decedent males had an increased incidence of small and/or flaccid testes as compared with decedent controls. Microscopic examination revealed a corresponding increased incidence of marked tubular atrophy at 100 ppm in decedent males (8/16; 50%) as compared with decedent controls (3/17; 18%); however, this increase was not statistically significant and did not show a dose-response when considering the average severity ranking for the decedent males. When considering all 100 ppm males (decedent + terminal), macroscopic examination did not reveal an increased incidence of small and/or flaccid testes, but the overall severity of testicular tubular atrophy was increased in high-dose rats (3.1) as compared with controls (2.6). Because testicular atrophy was also an effect noted in male rats following dietary treatment with 100 or 1000 ppm in another chronic toxicity/carcinogenicity study (MRID 42248620), it is considered an effect of treatment in this study. The increased relative testes and epididymides weights in high-dose males at study termination is of marginal biological significance (+17%), but was not accompanied by an increased incidence of benign testicular interstitial cell tumors.

High-dose females had significantly increased relative liver weights as compared with controls, but no corresponding hepatic histopathological changes were noted. Although adenomatous hyperplasia and alveolar epithelialization of the lung and exocrine acinar atrophy of the pancreas were noted in treated males and/or females, the lung lesions occurred only in a few animals and did not attain statistical significance, and the pancreatic lesion was not related to dose. Therefore, although these effects were observed in another chronic toxicity study in rats (MRID 42248620), these lesions were not considered an effect of treatment in the present study.

No treatment-related neoplastic lesions were observed.

Therefore, a LOAEL of 100 ppm (3.9 mg/kg/day) was identified for males based on increased testicular atrophy, with a corresponding NOAEL of 50 ppm (1.9 mg/kg/day). A LOAEL was not identified for females, and the NOAEL was therefore ≥100 ppm (4.9 mg/kg/day).

Although the testicular effects in the high-dose males are marginal and no adverse effects were observed in females, the doses selected in this study are considered acceptable in view of the adverse toxic effects observed at 100 and 1000 ppm in the combined chronic toxicity/oncogenicity study (MRID 42248620).

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B. STUDY DEFICIENCIES

Study deficiencies include: lack of data in this study (MRID 44839901) to verify acceptance of homogeneity and stability analyses of test diets, summary table of clinical signs was not provided, and the summary tables of body weight and food consumption data did not contain the standard deviations associated with the means for easy viewing by the reviewer.

APPENDIX

B-1216: Four-week Toxicity Study in Dietary Administration to CD Rats

Study Type: Range-finding feeding study (non-guideline) in rats

Test Material: Fluazinam; B-1216; IKF-1216; PP192; purity 96.3%; Lot No. # 8203

Citation: Broadmeadow, A. (1983). B-1216: Four-week toxicity study in dietary administration to CD rats. Life Science Research, Stock, Essex, CM4 9PE, England. Document No. 82/ISK035/544. May 23, 1983. MRID 44807213. Unpublished.

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

Objective: The objective of this study (MRID 44807213) was to assess the potential toxic effects of B-1216 to rats following dietary administration of 0, 10, 50, 250, or 3000 ppm for four weeks.

Methods:

Test animals: Sprague-Dawley CD rats approximately 5-6 weeks old, weights ranging from 101-153 g for males and 86-131 g for females. Received from Charles River (Margate, England). Individually housed.

Group size: 10 rats/sex/dose group

Test diet concentrations: 0, 10, 50, 250, or 3000 ppm; compound consumption time-weighted average (calculated by reviewer): 1.0, 5.1, 26.4, or 301.9 mg/kg/day for males; 1.1, 5.3, 25.9, or 308.9 mg/kg/day for females

Experimental protocol: The experimental protocol is generally the same as that described in the chronic toxicity study (MRID 44839901).

Diet preparation and analysis: The B-1216 was incorporated into powdered Laboratory Animal Diet No. 2 (Spratts Patent, Barking, England) weekly by initial preparation of the high-dose group concentration, and intermediate- and low-dose test diets were prepared by serial dilution from these diets. Samples were taken from six positions from the highest and lowest concentration test diets prepared for Week 5 (when the animals were awaiting necropsy). Stability of the test diets was determined by pooling the unused portion of the homogeneity samples for the highest and lowest concentration test diets.

Methods: Animals were observed twice daily for clinical signs. Food consumption and food conversion (mean food consumption/mean body weight gains) were determined weekly, water consumption measured for Weeks 1 and 4, and body weights were recorded on the commencement of treatment twice/week thereafter. Ophthalmoscopic examinations were conducted on all rats before treatment commenced and after 3 weeks of treatment. Blood was drawn for hematology and limited clinical chemistry analyses and urine collected for urinalysis from ten male and ten female rats before

the commencement of treatment and from all animals after 3 weeks of treatment. Macroscopic examination was conducted on all animals at necropsy, organ weights were recorded, tissues preserved for histological examination, and the brain, heart, kidneys, liver, lungs, marrow smear, spleen, and testes from all animals were examined microscopically.

Results:

Test material homogeneity and stability: The mean concentrations of the 10 and 3000 ppm test diet samples taken for homogeneity analyses were 9.9 ± 2.1 ppm and 2830 ± 102 ppm, respectively, with corresponding coefficients of variation (calculated by reviewer as standard deviation/mean x 100%) of 21% and 3.6%, respectively. The results of the stability analyses at Day 0, 6, and 14 revealed concentrations 9.9, 9.3, and 9.2 ppm for the 10 ppm group, respectively, and 2830, 2930, and 3030 ppm for the 3000 ppm group, respectively.

Toxicity:

3000 ppm - No animals died during the treatment period, and no treatment-related clinical signs were observed. Treatment-related differences in 3000 ppm males and females as compared with controls included:

decreased body weight gain for days 0-28 (males: 86% of controls, $p<0.01$; females: 75% of controls, $p<0.001$); decreased final body weights (males: 90% of controls, $p<0.01$; females: 88% of controls, $p<0.01$)

decreased food consumption over weeks 1-4 (males: 89% of controls; $p<0.01$; females: 86% of controls, $p<0.001$);

decreased hemoglobin concentration (males; females)

increased serum phospholipid (males: +22%, $p<0.01$; females: +40%, $p<0.001$) and total cholesterol (males: +35%,$p<0.001$; females: +47%, $p<0.001$)

increased absolute liver weight (males: +15%, $p<0.01$; females: +23%, $p<0.001$) and relative liver weight (males: +28%, $p< 0.001$; females: 41%, $p<0.001$)

changes in liver histopathology: increased incidence of periacinar hypertrophy in males: 4/10 treated males vs. 1/10 control males

increased incidence of occasional hepatocytic single cell necrosis with mononuclear infiltration in females: 6/10 treated females vs. 0/10 control females

Other changes noted but of questionable toxicological significance included decreased alanine aminotransferase in males and females (71% and 79% of controls, respectively), and in females decreased platelet count (87% of controls) and absolute ovary weights (85% of controls).

250 mg/kg/day - No animals died during the treatment period, and no treatment-related clinical signs were observed. Treatment-related differences in 250 ppm males and/or females as compared with controls included:

decreased body weight gain for days 0-28 in females (87% of controls, $p<0.05$)

decreased food consumption over weeks 1-4 in females (93% of controls; $p<0.05$)

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increased serum phospholipids in females (+17%, $p<0.05$) and total cholesterol in males (+18%, $p<0.005$) and females (+25%, $p<0.01$)
increased relative liver weight in females (+17%, $p<0.001$)
changes in liver histopathology: increased incidence of periacinar hypertrophy in males: 3/10 treated males vs. 1/10 control males

Of questionable toxicological significance was decreased alanine aminotransferase activity in males (83% of controls).

50 or 10 ppm - There were no clinical signs of toxicity and no mortality. No significant changes were observed in mean body weight or body weight gains. Gross necropsy and microscopic examination were unremarkable.

Conclusions: The study author concluded that 250 ppm was a LOAEL based on the hepatic changes including increased serum phospholipids and total cholesterol, an increased liver: body weight ratio in females, and increased incidence of periacinar hypertrophy in males. The NOAEL was 50 ppm. The decreases in body weight and food consumption were not considered to be toxicologically significant, but rather due to a non-specific effect such as unpalatability.

The NOAEL was 50 ppm (5.1 mg/kg/day for males; 5.3 mg/kg/day for females). The LOAEL was 250 ppm (26.4 mg/kg/day for males; 25.9 mg/kg/day for females).

This range-finding study is classified as **Acceptable/Non-Guideline**. It does **not** satisfy the Subdivision F guideline requirement for a subchronic oral toxicity study [OPPTS 870.3100 (82-1)] in rats.

Important Note:

In an addendum to this study (Huntingdon Life Sciences Ltd., England; August 27, 1998), slides of the brain for all control and high dose (3000 ppm) male and female rats in this study were microscopically re-examined for white matter vacuolation. There was no effect of treatment on the incidence or degree of white matter vacuoloation in the brain of rats of the high dose group (3000 ppm), compared with controls.

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