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DATA EVALUATION REPORT

FLUAZINAM

STUDY TYPE: CHRONIC ORAL TOXICITY (CAPSULE) - DOG
[OPPTS 870.4100 (§83-1b)]
MRID: 42270603, 44807219

Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
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Prepared by

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Work Assignment No. 99-51K

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DATA EVALUATION RECORD

STUDY TYPE: Chronic Oral Toxicity (Capsule) - Dog [OPPTS 870.4100 (§83-1b)]

DP BARCODE: D258235 SUBMISSION CODE: S561478
P.C. CODE: 129098 TOX. CHEM. NO.: None (new chemical)

TEST MATERIAL (PURITY): Fluazinam (95.3% w/w)

SYNONYMS: 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine; B1216; IKF1216; PP192.

CITATION: Broadmeadow, A. (1987) Fluazinam technical (B1216): 52-week toxicity study in oral administration to beagle dogs. Life Science Research (Eye, Suffolk, England), Laboratory report (document) number 86/ISK055/512, April 7, 1987. MRID 42270603. Unpublished.

Broadmeadow, A. (1998) Addendum to report no.86/ISK055/512: B-1216: 52-week toxicity study in oral administration to beagle dogs. Huntingdon Life Sciences Ltd. (Huntingdon, England), Laboratory report (document) number 86/ISK055/512 Addendum 1. September 25, 1998. MRID 44807219. Unpublished.

SPONSOR: Ishihara Sangyo Kaisha Ltd., 2-3-1, Nishi-Shibukawa, Kusatsu, Shiga 525, Japan

EXECUTIVE SUMMARY: In a chronic oral toxicity study (MRIDs 42270603, main study and 44807219, addendum), Fluazinam (Lot No. 8412-20, 95.3% purity) was administered to groups of six male and six female beagle dogs/dose for 52 weeks at doses of 0, 1, 10, or 50 mg/kg/day in gelatin capsules.

No animals died as a result of treatment. The most notable clinical signs were increased incidence of salivation and nasal dryness, mainly in the high-dose dogs but nasal dryness was also slightly increased in females at 10 mg/kg/day. Body weight was mildly decreased at high dose (-4%, males and -9%, females; not analyzed statistically), and total body weight gain was significantly reduced (29%, $p<0.05$; -13% when calculated as a percentage of initial body weight) only in females but was also lower in males (-19%; -9% as a percentage of initial body weight). Hematocrit, hemoglobin, and RBC counts of high-dose dogs were consistently lower (8-17%; $p<0.05$, 0.01, or 0.001) than controls throughout the treatment period, and WBC counts were elevated (32-64%, $p<0.05$ or 0.001) at study end (these findings considered treatment-

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related but not biologically significant). Alkaline phosphatase was significantly increased (52-183%; $p<0.05$, 0.01, or 0.001) in high-dose dogs throughout the treatment period.

Absolute liver weight (males, 37%; females, 16%; $p<0.05$) and the liver/body weight ratio (males, 45%; females, 47%; $p<0.01$) were increased in high-dose dogs. In the reexamination of brain and spinal cord tissues, incidence of vacuolation of white matter in the brain was increased in both sexes at the high dose (6/6 animals/sex affected vs. 2-4/6, controls), along with increased severity (1.5-2.17 vs. 1.0, controls). In addition, vacuolation of the white matter of the spinal cord was seen in high-dose females (4/6 affected vs. 0, controls). An increase in liquefied GI tract contents and incidence/severity of stomach mucosal lymphoid hyperplasia was seen in mid- and high-dose dogs of both sexes, although in females, neither incidence nor mean severity of the hyperplasia at these dose levels showed a dose-related increase.

The LOAEL (threshold) is 10 mg/kg/day for both male and female dogs, based on marginal increases in the incidence of nasal dryness in females and the incidence/severity of gastric lymphoid hyperplasia in both sexes. The NOAEL is 1 mg/kg/day.

This chronic toxicity study is classified as **Acceptable/guideline** and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100 (§83-1b)] in dogs. No major deficiencies were noted in this study.

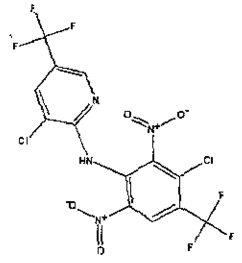
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided for MRID 42270603 and MRID 44807219.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Fluazinam, tech.

   Description: Pale yellow crystalline powder
   Lot/Batch #: 8412-20
   Purity: 95.3%
   Stability of compound: Stable for duration of study (stored in dark, room temperature)
   CAS #: 79622-59-6
   Structure:



2. Vehicle and/or positive control

The test substance was contained in gelatin capsules. Negative control dogs received an empty capsule. No positive controls were used in this study.

3. Test animals

Species: dog
Strain: beagle
Age and weight at study initiation: 19-25 weeks old; females, 6.7-11.0 kg; males, 8.5-12.8 kg.
Source: Animal Breeding Unit, Imperial Chemical Industries plc, Pharmaceuticals Division, Alderley Park, Cheshire, England
Housing: individually in kennels
Diet: dry pelleted Laboratory diet A (Special Diet Services, Ltd., Witham, Essex, England), 350 g offered daily prior to dosing (any remaining removed next day)
Water: municipal water, *ad libitum.*
Environmental conditions (all reported to be within appropriate range):
Temperature: not specified
Humidity: not specified
Air changes: not specified
Photoperiod: 12 hour light/dark
Acclimation period: 29 days

B. STUDY DESIGN

1. In life dates

Start: July 4, 1985; end: July 8, 1986

2. Animal assignment

Dogs were ranked by body weight and litter within each sex, then assigned by litter to the test groups in Table 1 using sequences from randomly derived Latin squares.

TABLE 1: Study design

Test Group	Dose to Animal (mg/kg/day)	Number of Animals	
		Male	Female
Control	0	6	6
Low	1	6	6
Mid	10	6	6
High	50	6	6

Data taken from p. 8, MRID 42270603.

3. Dose selection rationale

Doses were selected based on data held by the sponsor and on results of previous studies performed at the same laboratory. These data were not included in MRIDs 42270603 or 44807219.

4. Diet preparation and analysis

The test material was milled to ≤1 mm and administered daily in gelatin capsules loaded for individual animals with dosages based on the most recent body weight and adjusted for a purity of 95.3%..

Results -
Homogeneity analysis: Not applicable(test material administered in gelatin capsules).

Stability analysis: Not applicable; the test material was administered in gelatin capsules. The test material itself was stable under the storage conditions, as a sample from the bulk container returned to the sponsor for analysis after the study ended had a fluazinam content of 95.2%, compared to 95.3% prior to the study start.

Concentration analysis: Not applicable (test material administered in gelatin capsules.

5. Statistics

The significance of intergroup differences in body weight change, hematology, bone marrow (myeloid to erythroid ratio), blood chemistry, and quantitative urinalysis was determined with Student's t-test using a pooled error variance. The significance of intergroup differences in the incidences of histopathological abnormalities was determined by Fisher's exact test. The significance of intergroup differences in organ weights was determined by Dunnett's test. For all tests the minimum level of significance was $p<0.05$.

C. METHODS

1. Observations

Dogs were observed at least once daily for gross clinical and behavioral abnormalities. In addition, each animal received a detailed weekly examination where any chronic conditions were noted. All dogs were examined by a veterinarian before treatment began and at treatment weeks 12, 24, 38, and 50.

2. Body weight

Dogs were weighed weekly before feeding during the acclimation and treatment periods. Each dog was weighed prior to necropsy regardless of the feeding cycle.

3. Food consumption and food efficiency (food conversion ratio)

Food consumption was determined daily by weighing any food residues and spillages prior to giving the next meal. Food conversion ratios for each dose group were calculated as weight of food consumed (g/week) ÷weight gain (g/week).

4. Water consumption

Water consumption was measured twice pretest and weekly thereafter.

5. Ophthalmoscopic examination

Both eyes of each dog were examined by indirect ophthalmoscopy before treatment began and at treatment weeks 12, 24, and 50. The retinas were photographed (including the tapetum of both eyes of each dog).

6. Blood was collected from the jugular vein of each dog (following an overnight fast) before treatment began and after treatment weeks 12, 23, and 50 for hematology and clinical analysis. The CHECKED (X) parameters were examined.

a. Hematology

X		X	
x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	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	Erythrocyte sedimentation rate
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Required for chronic studies based on Subdivision F Guidelines

The study author noted that duplicate bone marrow smears were prepared at terminal sacrifice from femurs of the first 2 males of the control and mid dose groups and the first 3 males of the low and high groups. Because the smears were of poor quality in a preliminary examination and slides were misplaced (one from each group), samples were obtained from all of the females and the remaining males from the iliac crest shortly before terminal sacrifice. One hundred nucleated cells/smear were counted.

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*		Globulins
x	Sodium*	x	Glucose*
		x	Total bilirubin
	ENZYMES	x	Total serum protein (TP)*
x	Alkaline phosphatase (ALK)		Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
x	Creatine phosphokinase		
	Lactic acid dehydrogenase (LDH)		
x	Serum alanine amino-transferase (also SGPT)*		
x	Serum aspartate amino-transferase (also SGOT)*		
x	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Required for chronic studies based on Subdivision F Guidelines

6. Urinalysis

Urine was collected overnight from fasted and water-deprived animals prior to treatment and at treatment weeks 12, 24, and 50. 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
		x	Total reducing substances

* Required for chronic studies based on Subdivision F Guidelines

7. Sacrifice and pathology

All dogs survived to study termination, at which time they were sacrificed by i.v. sodium pentobarbitone anesthesia and exsanguination, and subjected to gross pathological examination. The CHECKED (X) tissues were collected from all animals and examined histologically. The (XX) organs, in addition, were weighed.

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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*	xx	Thymus*		
x	Ileum*				**GLANDULAR**
x	Cecum*		**UROGENITAL**	xx	Adrenal gland*
x	Colon*	xx	Kidneys*+		Lacrimal gland
x	Rectum*	x	Urinary bladder*	x	Mammary gland*
xx	Liver*+	xx	Testes*+	xx	Parathyroids*++
x	Gall bladder*	x	Epididymides	xx	Thyroids*++
x	Pancreas*	xx	Prostate		
			Seminal vesicle		**OTHER**
	RESPIRATORY	xx	Ovaries*+	x	Bone*
x	Trachea*	xx	Uterus*	x	Skeletal muscle*
xx	Lung*			x	Skin*
	Nose				All gross lesions and masses*
	Pharynx				
	Larynx				

*Required for chronic studies based on Subdivision F Guidelines
+Organ weight required in chronic studies. ++Organ weight required for non-rodent studies.

The left eye from each dog was processed for electron microscopy by the Central Toxicology Laboratory at ICI plc. Phosphate-buffered 3% glutaraldehyde was injected into the anterior chamber, eyes were removed and additional fixative was injected into the vitreous humor. After 2 or more days in fixative, eyes were dehydrated, embedded in Araldite resin and stained with uranyl acetate and lead citrate. Semithin sections for light microscopy and ultrathin sections for electron microscopy were prepared.

In the Addendum to the main study report (MRID 44807219), brain and spinal cord tissues from all dogs were re-examined (in 1998 by the Pathology Department of Huntingdon Life Sciences) to determine whether vacuolation, as reported in two mouse carcinogenicity studies at doses ≥107 mg/kg/day(MRIDs 44807220 and 44807221;see reviews in this HED Doc. No.), was present. The re-examination was conducted using the same criteria for severity grading used in the mouse studies.

II. RESULTS

A. OBSERVATIONS

1. Toxicity
Salivation was occasionally seen in animals from all groups, but slightly more frequently at 50 mg/kg/day. It usually occurred between 1-4 hours post-dosing, and, on isolated occasions, persisted until the following day. In control, low and mid dose groups, this finding was very sporadic, generally observed once or twice during the study in 1-3 animals/sex/dose group (with the exception of low dose male #596,

observed 8 times). At high dose, incidence was still sporadic but was observed in all males (total of 59 incidences) and 4 females (39 incidences). Except for 2 females and 1 male affected only on 1-2 days, other animals were affected on 5-22 days. Nasal dryness was also noted in all groups, especially during the first 13 weeks of treatment; however, the association of this finding with treatment was less clear because it was observed on some occasions in all groups and in almost all animals. Dryness was most frequent in females at 10 or 50 mg/kg/day (a total of 135 and 121 incidences, respectively, among 6 animals/dose group compared to 28 incidences among 5 control animals; 2-3 females in each group showing greatest frequency of effect), but high-dose males also had a marginally increased incidence (210 incidences in 6 animals) compared to controls (62 incidences in 5 animals).

2. Mortality

All dogs survived to the scheduled termination date.

B. BODY WEIGHT AND WEIGHT GAIN

Selected mean body weights and weight gain (cumulative) are show below in Table 2:

TABLE 2: Selected group mean body weight and weight gain in dogs given Fluazinam for 52 weeks

Parameter	Study Day	Dose (mg/kg/day)			
		0	1	10	50
Males					
Mean body weight, kg	0	10.6	10.8	10.5	10.7
	56	12.3	12.4	12.2	12.1 (98)
	92	13.2	13.1	13.1	12.5 (95)
	182	14.2	14.6	14.1	13.5 (95)
	273	14.4	14.2	14.4	13.6 (94)
	364	14.8	14.5	14.9	14.1 (95)
Mean weight gain, kg	0-92	2.6	2.3	2.6	1.8 (69)
	92-182	1.0	0.9	1.1	0.9 (90)
	182-273	0.2	0.3	0.3	0.2 (100)
	273-364	0.4	0.3	0.5	0.5 (120)
	0-364	4.2	3.7	4.5	3.4 (81)
Females					
Mean body weight, kg	0	9.3	9.1	9.1	9.3
	56	10.9	10.7	10.9	10.4 (95)
	92	11.5	11.3	11.4	10.8 (94)
	182	12.5	12.2	12.3	11.6 (93)
	273	12.9	12.4	12.7	11.7 (91)
	364	13.4	12.8	13.2	12.2 (91)
Mean weight gain, kg	0-92	2.3	2.4	2.5	1.7 (73)
	92-182	0.9	0.8	0.7	0.6 (67)
	182-273	0.4	0.2	0.4	0.2 (50)
	273-364	0.5	0.4	0.6	0.5 (100)
	0-364	4.1	3.8	4.1	2.9* (71)

Data taken from Table 4, pp. 56-59, MRID 42270603.*Statistically different from control group, $p<0.05$
[a] Numbers in parentheses are percent of control value, calculated by the reviewer

At 50 mg/kg/day, mild decreases in mean body weight (-4.7% in males and -9% in females; not evaluated statistically) were reported at study termination. This decrease was due to decreased weight gain during the first months of the study when the animals were growing more rapidly. Mean weight gain was statistically significantly decreased at termination in females (-29% less than controls, $p<0.05$); in males, gain was reduced (-19%) but the decrease was not statistically significant . When calculated as a percent of initial mean body weight, gain was -8% less than controls in males and -13% less in females. No effects on body weight or gain were observed at 1 or 10 mg/kg/day. Because decreased gain was observed in both sexes during the early portion of the study and was statistically significant in females, with resulting mild decreases in terminal body weight, the effect is considered treatment-related.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

There were no statistically significant differences in food consumption during the study. A single high-dose female left a small residue of food during most weeks of the first three months of treatment, and occasionally thereafter. Small residues were also left on a few occasions during the first three months of treatment by another high dose female and three high-dose males. With very few exceptions, the remaining dogs ate all the food offered during the treatment period.

2. Compound consumption

The compound was administered in gelatin capsules, with the amount given determined by the most recent body weight measurement.

3. Food efficiency

The study author calculated a food conversion ratio (weight of food consumed/unit gain in body weight) for the first 13 weeks of treatment. Group mean food conversion ratios for the high-dose dogs (males, 17.4; females, 18.5) were higher than controls (males, 12.3; females 13.9), indicating a reduced food efficiency. Low- and mid-dose dogs were unaffected.

4. Water consumption

No treatment-related alterations in water consumption were observed.

D. OPHTHALMOSCOPIC EXAMINATION

No treatment-related ocular lesions were found as a result of examination by indirect ophthalmoscopy or examination of retinal photographs. Also, examination of ultrathin sections of left eyes by electron microscopy and of semithin sections by light microscopy revealed no treatment-related ocular lesions. .

E. BLOOD WORK

1. Hematology

Selected group mean hematology values are given in Table 3. The hematocrit, hemoglobin, and erythrocyte values for dogs in the 50 mg/kg/day group were consistently lower than those of controls throughout the treatment period. In males, significant decreases occurred in hematocrit (9-11%, $p<0.05$ or 0.01), hemoglobin (7-9%, $p<0.05$) and RBC count (9-12%, $p<0.05$ or 0.01) at all sampling times. In females, significant decreases occurred in hematocrit (9-14%, $p<0.05$ or 0.001) and RBC count (10-17%, $p<0.05$ or 0.001) at all sampling times and in hemoglobin (8-13%, $p<0.05$ or 0.01) at weeks 12 and 23. WBC counts were significantly increased (29-64%, $p<0.05$, 0.01, or 0.001) in high-dose males at all sampling times and in high-dose females (30-41%, $p<0.05$) at weeks 23 and 50. WBC counts were also increased in mid-dose males (28%, $p<0.05$) at week 23 and mid-dose males (33%, $p<0.05$) and females (32%, $p<0.05$) at week 50. These changes are considered treatment-related because they were consistently observed in both sexes, but are marginal and probably not biologically significant. In the examination of bone marrow smears, the myeloid:erythroid ratio was increased in females at mid and high dose by 26% (statistically significant, $p<0.05$), along with decreases in the erythroid series in 2 females at these dose levels (vs. 0 in control and high dose groups; see Attachment 1). No significant decreases were observed in males. This increase was also of uncertain biological significance and not considered an adverse effect.

TABLE 3: Selected group mean hematology values for dogs given Fluazinam for 52 weeks

Parameter	Week	Dose (mg/kg/day)			
		0	1	10	50
Males					
Hematocrit (%)	0	44±2	41±2	44±4	43±2
	12	43±3	43±1	43±3	39±3** (91)[a]
	23	47±4	46±1	46±5	43±1* (91)
	50	46±4	44±5	43±3	41±3* (89)
Hemoglobin (g%)	0	14.1±0.8	13.6±0.5	14.3±1.3	14.0±0.9
	12	14.1±0.8	14.1±0.4	14.2±0.8	13.1±0.6* (93)
	23	15.9±1.3	15.5±0.6	15.7±1.5	14.6±0.4* (92)
	50	16.0±1.3	15.5±1.2	15.5±1.2	14.6±0.9* (91)
RBC (mil/cmm)	0	6.41±0.43	6.09±0.27	6.47±0.61	6.21±0.47
	12	5.92±0.37	5.89±0.12	5.94±0.40	5.37±0.37** (91)
	23	6.92±0.66	6.64±0.19	6.73±0.66	6.13±0.24* (89)
	50	6.80±0.63	6.50±0.68	6.37±0.49	5.99±0.37* (88)
WBC (1000/cmm)	0	13.4±1.8	14.7±2.9	14.1±1.4	14.6±2.7
	12	11.8±3.6	11.2±2.1	14.3±1.4	15.2±2.1* (129)
	23	12.4±2.2	15.2±3.7	15.9±1.2* (128)	17.0±2.0** (137)
	50	11.6±2.0	12.2±1.4	15.4±2.5* (133)	19.0±4.4*** (164)

TABLE 3: Selected group mean hematology values for dogs given Fluazinam for 52 weeks					
Parameter	Week	Dose (mg/kg/day)			
		0	1	10	50
Females					
Hematocrit (%)	0	41±2	42±3	42±2	42±2
	12	44±3	45±3	43±1	40±3* (91)
	23	50±5	48±3	46±2* (92)	43±2*** (86)
	50	44±4	46±3	43±3	40±1* (91)
Hemoglobin (g%)	0	13.5±0.6	13.7±1.0	13.6±0.6	13.3±0.8
	12	14.4±1.1	14.7±1.0	14.1±0.2	13.2±1.0* (92)
	23	16.6±1.5	16.0±0.8	15.6±0.9	14.4±0.9** (87)
	50	15.4±1.3	16.1±1.3	14.9±0.7	14.3±0.5 (93)
RBC (mil/cmm)	0	6.10±0.29	6.28±0.61	6.16±0.22	6.07±0.30
	12	6.15±0.31	6.22±0.49	5.87±0.24	5.47±0.45** (89)
	23	7.33±0.64	7.10±0.43	6.67±0.23* (91)	6.10±0.38*** (83)
	50	6.58±0.57	6.79±0.52	6.19±0.43	5.89±0.17* (90)
WBC (1000/cmm)	0	14.0±4.2	13.6±1.8	14.1±2.5	13.4±3.1
	12	12.1±2.1	12.7±1.4	13.1±1.3	14.4±3.3
	23	13.3±2.5	14.2±2.4	15.9±1.6	17.3±3.3* (130)
	50	13.0±2.8	15.1±2.2	17.1±2.5* (132)	18.3±4.9* (141)

Data taken from Tables 6A-6D, pp. 61-68, MRID 42270603.
*Statistically different from control group, $p<0.05$
**Statistically different from control group, $p<0.01$
***Statistically different from control group, $p<0.001$
[a] Numbers in parentheses are percent of control value, calculated by the reviewer

2. Clinical Chemistry

Selected group mean clinical chemistry changes are given in Table 4. Plasma alkaline phosphatase was significantly increased in high-dose males (89-183%, $p<0.001$) and females (52-88%, $p<0.05$ or 0.01) at all sampling times. Low and mid-dose animals were not affected. This increase correlated with increased liver weight in both sexes.

In addition, albumin levels were significantly reduced at week 23 in high dose males (13%, $p< 0.01$) and at weeks 23 and 50 in females (9-12%, $p<0.05$). Other statistically significant clinical chemistry changes included increased cholesterol (25%, $p<0.05$) in high-dose males at week 50, decreased glucose in high-dose males at weeks 12 (8%, $p<0.01$) and 23 (11%, $p<0.001$) and in low-, mid-, and high-dose females at week 23 (5%, $p<0.05$). Although they may have been related to treatment, these findings were marginal or not consistent throughout the study, they are not considered biologically significant.

TABLE 4: Selected group mean clinical chemistry changes in dogs given Fluazinam for 52 weeks					
Parameter	Week	Dose (mg/kg/day)			
		0	1	10	50
Males					
Alkaline phosphatase (IU/L)	0 12 23 50	84±15 64±10 52±10 39±16	94±20 70±11 60±16 40±9	96±13 82±12 73±17 53±13	103±19 121±39*** (189) 147±63*** (283) 109±53*** (279)
Albumin (g%)	0 12 23 50	3.1±0.1 3.3±0.6 3.1±0.2 3.2±0.2	3.1±0.2 3.2±0.4 3.0±0.1 3.4±0.3	3.0±0.1 2.9±0.2 3.1±0.1 3.1±0.1	3.1±0.2 3.0±0.2 2.7±0.3** (87) 2.9±0.3
Total cholesterol (mg%)	0 12 23 50	178±24 159±35 172±30 159±30	153±25 162±26 167±20 150±18	154±21 154±19 163±34 138±19	182±27 199±56 201±64 198±43* (125)
Females					
Alkaline phosphatase (IU/L)	0 12 23 50	90±27 64±19 57±22 46±19	94±12 66±4 52±5 39±17	93±16 68±12 61±8 51±10	90±25 97±32* (152) 107±39** (188) 75±30* (163)
Albumin (g%)	0 12 23 50	3.1±0.2 3.1±0.3 3.4±0.2 3.4±0.1	3.0±0.2 3.0±0.2 3.3±0.3 3.4±0.2	3.1±0.2 3.2±0.2 3.2±0.2 3.2±0.2	3.1±0.3 3.1±0.2 3.0±0.4* (88) 3.1±0.2* (91)
Total cholesterol (mg%)	0 12 23 50	161±18 142±20 157±12 169±31	149±11 156±24 167±24 152±33	157±27 147±23 167±19 187±46	171±22 164±30 192±58 175±37

Data taken from Tables 8A-8D, pp. 70-85, MRID 42270603.*Statistically different from control group, $p<0.05$
**Statistically different from control group, $p<0.01$
***Statistically different from control group, $p<0.001$
[a] Numbers in parentheses are percent of control value, calculated by the reviewer

F. URINALYSIS

There were no treatment-related changes in urinalysis parameters.

G. SACRIFICE AND PATHOLOGY

1. Organ weight

Treatment-related effects on absolute and relative liver weights were observed. Absolute liver weights were significantly increased in males (36.8%, $p<0.05$) and females (16.2%, $p<0.05$) of the 50 mg/kg/day group, compared to controls (Table 5). Liver weights relative to body weight were also increased in dogs of that group (males: 45.1%, $p<0.01$; females: 47.4%, $p<0.01$). Slight increases (<15%) in liver weight at 10 mg/kg/day in males and females were not statistically significant and not associated with increased alkaline phosphatase.Both absolute and relative ovary weights were decreased only in low dose females (58.7% and 55.0% of controls, respectively, $p<0.05$) and were therefore not considered treatment-related.

TABLE 5: Group mean absolute and relative liver weights in dogs given Fluazinam for 52 weeks

Parameter	Dose (mg/kg/day)			
	0	1	10	50
Males				
Mean terminal body weight (kg)	15.4167±1.5303	15.0667±0.9158	15.5333±1.1237	14.4333±0.7942
Absolute liver weight (g) Liver/body weight ratio (%)	468±38 3.06±0.36	441±28 2.94±0.25	509±60 3.27±0.29	640±108* (137)[a] 4.44±0.78** (145)
Females				
Mean terminal body weight (kg)	14.0353±.9459	13.3167±1.7982	13.8667±1.4109	12.7500±2.0964
Absolute liver weight (g) Liver/body weight ratio (%)	431±61 3.08±0.42	416±67 3.13±0.31	491±93 3.53±0.49	501±128* (116) 4.54±0.49** (147)

Data taken from Table 10A-B, pp. 102-107, MRID 42270603.
*Statistically different from control group, $p<0.05$
**Statistically different from control group, $p<0.01$
[a]Numbers in parentheses are percent of control value, calculated by the reviewer

2. Gross pathology

The incidence of liquefied contents in the jejunum was increased in both sexes compared to controls. For males, 1/6, 2/6, 3/6, and 4/6 dogs were affected in the 0, 1, 10, and 50 mg/kg/day groups respectively; in females, 2/6, 1/6, 3/6, and 4/6 dogs were affected. Two males in the high dose group had liquefied contents throughout the entire g.i. tract, and another high-dose male had liquefied contents in the stomach, duodenum, jejunum, and ileum. Two high-dose females also had the entire g.i. tract affected. The study author considered treatment to have contributed to the occurrence of this finding, although no alteration in fecal consistency was observed.

The gall bladders of one male and three females in the high-dose group contained dark, free masses, and were distended in the male and one of the females. Dark punctate masses were also found in the gall bladder of one control female, but there were no associated microscopic effects. These gallbladder findings were not considered treatment-related. One high-dose female had an enlarged liver which correlated with increased liver weight. One high-dose male had numerous pale masses of firm amorphous tissue throughout the pyloric mucosa, which the study author considered possibly related to the gastric lymphoid hyperplasia (apparently not confirmed by microscopic evaluation). The same dog also had a firm, pale mass on the lateral surface of a testis.

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3. Microscopic pathology

a) **Non-neoplastic** – There was an increase in the incidence and severity of mucosal lymphoid hyperplasia in the stomachs of males receiving 10 or 50 mg/kg/day (Table 6). In females, a similar effect was indicated at 10 mg/kg/day, but at 50 mg/kg/day the incidence was similar to that in controls and severity only slightly increased due to 1 female with moderate severity.

TABLE 6: Microscopic changes in stomachs of dogs given Fluazinam for 52 weeks[1]

Stomach: Mucosal lymphoid hyperplasia	Dose (mg/kg/day)			
	0	1	10	50
Males (n=6)				
Total	3 (1.0)[2]	2 (1.0)	6 (2.66)	6 (2.33)
minimal	3	2	0	1
slight	0	0	2	2
moderate	0	0	4	3
Females (n=6)				
Total	4 (1.25)	6 (1.33)	6 (2.16)	4 (1.50)
minimal	3	4	1	3
slight	1	2	3	0
moderate	0	0	2	1

1 Data taken from text table, p. 37, MRID 42270603.
2 Numbers in parentheses are the mean severity of the lesion at that dose level.

In subsequent long-term mouse carcinogenicity studies (MRIDs 44807220 and 44807221), treatment-related vacuolation of white matter in the brain and cervical spinal cord was noted in mice of the dosed groups. In view of this finding, the study sponsor requested that slides for the brain and spinal cord from all dogs in the present study be examined for white matter vacuolation using the same criteria for severity as the mouse studies. The results are presented in Table 7. Males receiving 50 mg/kg/day had a statistically significantly ($p<0.01$) increased incidence (6/6 compared to 3/6 controls) of white matter vacuolation in the cerebrum, and an increased incidence (6/6 compared to 3/6 controls) of white matter vacuolation in the cerebellum/pons/ medulla/midbrain. Females receiving 50 mg/kg/day had a significantly ($p<0.05$) increased incidence (6/6 compared to 2/6 controls) of white matter vacuolation in the cerebrum and an increased incidence (6/6 compared to 4/6 controls) of white matter vacuolation in the cerebellum/pons/medulla/midbrain. Severity of the lesion in the cerebrum of both sexes was increased; in the females, severity in the cerebellum/pons/medulla/midbrain was also slightly increased. The high-dose females also had a statistically significant ($p<0.05$) increase (4/6 compared to 0/6 controls) of trace to minimal vacuolation of white matter in the spinal cord, a finding not observed in males.

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TABLE 7: Brain and spinal cord findings in dogs given Fluazinam for 52 weeks

Parameter	Dose (mg/kg/day)			
	0	1	10	50
Males (n=6)				
Cerebrum				
No abnormalities detected	3	3	4	0
Vacuolation of white matter - total	3 (1.0)[1]	3 (1.0)	2 (1.0)	6 (1.83)
Trace	3	3	2	1
Minimal	0	0	0	5**
Moderate	0	0	0	0
Cerebellum/pons/medulla/midbrain				
No abnormalities detected	3	3	4	0
Vacuolation of white matter - total	3 (1.0)	3 (1.0)	2 (1.0)	6 (1.0)
Trace	3	3	2	6
Minimal	0	0	0	0
Spinal cord				
Vacuolation of white matter - total	0	0	0	0
Trace	0	0	0	0
Minimal	0	0	0	0
Females (n=6)				
Cerebrum				
No abnormalities detected	4	4	3	0*
Vacuolation of white matter - total	2 (1.0)	2 (1.0)	3 (1.0)	6* (2.17)
Trace	2	2	3	1
Minimal	0	0	0	3
Moderate	0	0	0	2
Cerebellum/pons/medulla/midbrain				
No abnormalities detected	2	2	0	0
Vacuolation of white matter - total	4 (1.0)	4 (1.0)	6 (1.0)	6 (1.5)
Trace	4	4	6	3
Minimal	0	0	0	3
Spinal cord				
Vacuolation of white matter - total	0	0	0	4*
Trace	0	0	0	3 (1.25)
Minimal	0	0	0	1

Data taken from text table, p. 7, MRID 44807219 (Addendum to study report)
1 Values in parentheses indicate mean severity of lesion. 1 = trace; 2 = minimal; 3 = moderate; 4 = severe.
*Significantly different from control, p<0.05
**Significantly different from control, p<0.01

b) **Neoplastic** – There was no evidence that treatment caused a neoplastic response in any group of animals.

III. DISCUSSION

A. DISCUSSION

In general, the reviewer agreed with the conclusions of the study author. No animals died during this study. The most notable clinical signs were increased salivation and nasal dryness in dogs of both sexes. With the exception of nasal dryness in mid-dose females, these signs were increased in the high-dose dogs, and were minimal in nature. Body weight was decreased by 4.7% in males and 9% in females (data not analyzed statistically). Cumulative body weight gain for the whole treatment period was decreased in both sexes (19%, males and 29%, females), but was significantly reduced ($p<0.05$) only in females. When calculated as a percentage of the initial body weight, cumulative gain was decreased by8% and 13% in males and females, respectively. Food conversion ratios calculated by the study author for the first 13 weeks were increased for high-dose dogs compared to controls, indicating a reduced food efficiency. The high dose may have minimally affected appetite, but no firm conclusion can be drawn due to the restricted amount of food offered. There were no treatment-related ocular effects.

At high dose, hematocrit, hemoglobin, and RBC counts of high-dose dogs were consistently lower (males: 8-12%, $p<0.05$ or 0.01; females: 8-17%, $p<0.05$, 0.01, or 0.001) than those of controls throughout the treatment period. Bone marrow smears taken at necropsy showed a marginal increase in the myeloid/erythroid ratio in mid- and high-dose females. WBC counts were elevated (32-64%, $p<0.05$ or 0.001) in mid and high-dose dogs of both sexes at the end of the treatment period. These changes are considered treatment-related, but due to their small magnitude, are not considered adverse. Plasma alkaline phosphatase was significantly increased (52-183%, $p<0.05$, 0.01, or 0.001) in high-dose dogs throughout the treatment period, indicating a slight treatment-related effect on the liver. Changes in albumin and cholesterol may have been related to treatment, but are considered minimal. Urinalysis was unremarkable.

Absolute liver weight (males, 37%; females 16%; $p<0.05$) and the liver/body weight ratio (males, 45%; females, 47%; $p<0.01$) were both increased in high-dose dogs (one female showed grossly visible enlarged liver), which, along with the increased alkaline phosphatase levels (and possibly decreased albumin and increased cholesterol), indicates a slight treatment-related effect on the liver. No other organ weights showed a treatment-related effect. The increased incidence and extent of liquefied contents of the gastrointestinal tract in mid- and high-dose dogs of both sexes may have been increased by treatment, but this finding also occurred in the control group, and the study author stated it is commonly seen in dogs at that facility. The study author also stated that no effect on fecal consistency was seen during the study and that there were no microscopic correlates within the intestinal tract. There was an increased incidence and severity of mucosal lymphoid hyperplasia of the stomach found in mid- and high-dose dogs that may have been related to treatment, although there was no clear dose-response relationship at these 2 dose levels for incidence in females or severity in both sexes. Gall bladder findings (masses and distention) in animals of the high-dose group are not clearly treatment-related, and there were no microscopic correlates. The increased incidence and severity of vacuolation of white matter in the brain of both sexes of high-dose dogs are considered treatment-related, as is the vacuolation of white matter in the spinal cord of high-dose females. The cerebrum appeared to be the most sensitive region of the CNS and females appeared to be more sensitive than males, based on increased severity and effects in the spinal cord. In addition, females showed more pronounced effects on body weight/weight gain and liver weight.

Under the conditions of this study, the chronic oral toxicity LOAEL (threshold)for dogs of both sexes is 10 mg/kg/day, based on slightly increased incidence of nasal dryness in females and incidence and severity of stomach lymphoid proliferation in both sexes. The NOAEL is 1 mg/kg/day.

This chronic oral study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for a chronic oral toxicity study [OPPTS 870.4100 (§83-1b)] in dogs.

B. STUDY DEFICIENCIES

(1) A minor deficiency of this study is that the humidity, temperature, and air change frequency of the animal room were not reported. However, the study author did indicate that they were recorded, and there was no "exceptional fluctuation." (2) It appeared that all gross lesions and masses were not examined (eg., the grossly visible white areas in the stomach were apparently not evaluated microscopically).

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ATTACHMENT 1

Summary of findings of bone marrow smear
STUDY REPORT TABLE 7, p. 69

MRID 42270603

42270603.der
RAB2400:fluazi22.050

Bone marrow - summary of findings for animals killed after 52 weeks of the treatment period

Group	:	1	2	3	4
Compound	:	Control	------- B-1216 -------		
Dosage (mg/kg/day)	:	0	1	10	50

Group /sex	Myeloid: erythroid ratio	No. of smears examined	Cellularity and cell composition normal	Decrease in erythroid series
1M	2.2	4	3	1
SD	0.8			
2M	2.7	4	3	1
SD	1.2			
3M	2.4	4	2	2
SD	0.8			
4M	2.6	3	1	2
SD	0.5			
1F	1.9	6	6	0
SD	0.2			
2F	1.9	6	6	0
SD	0.3			
3F	2.4[a]	6	4	2
SD	0.6			
4F	2.4[a]	6	4	2
SD	0.4			

Myeloid: erythroid ratios are mean values; other columns record incidence

SD Standard deviation
a Significantly different from controls, $P<0.05$

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DATA EVALUATION REPORT

FLUAZINAM TECHNICAL (B1216)

STUDY TYPE: MULTIGENERATION REPRODUCTION - RAT [OPPTS 870.3800 (§83-4)]

MRIDs 42248619, 42208406, 42248618

Prepared for

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

Prepared by

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
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Primary Reviewer: Tessa L. Long, Ph.D.	Signature: Date:	Tessa L. Long MAR 0 8 2000
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Disclaimer

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

Managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under Contract No. DE-AC05-96OR22464.

FLUAZINAM **Reproduction Study [OPPTS 870.3800 (§83-4)]**

EPA Reviewer: E. Budd, M.S. Edwin R. Budd, Date 4/14/00
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: M. Copley, D.V.M., D.A.B.T. M Copley, Date 7/29/00
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Multigeneration Reproduction - Rat; OPPTS 870.3800 [§83-4]

DP BARCODE: D258235 SUBMISSION CODE: S561478
P.C. CODE: 129098 TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): Fluazinam technical (95.3 % a.i.)

SYNONYMS: B1216, IKF-1216, PP192

CITATION: Tesh, J.M., C.R. Willoughby, J.S.L. Fowler. (1987) Fluazinam Technical (B1216): Effects upon reproductive performance of rats treated continuously throughout two successive generations. Life Science Research, Eye, Suffolk IP23 7PX, England. Document No. 87/ISK068/097. December 14, 1987. MRID 42248619. Unpublished.

Tesh, J.M., et al. (1985) Fluazinam Technical (B1216): Effects upon reproductive function and performance in rats 1. Dose range finding study. Life Science Research, Eye, Suffolk IP23 7PX, England. Document No. 84/ISK043/547. January 28, 1985. MRID 42208406. Unpublished.

Tesh, J.M., et al. (1986) Fluazinam Technical (B1216): Effects upon reproductive function and performance in rats 1. Second Dose range finding study. Life Science Research, Eye, Suffolk IP23 7PX, England. Document No. 85/ISK050/295. June 3, 1986. MRID 42248618. Unpublished.

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

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

EXECUTIVE SUMMARY: Technical grade fluazinam (95.3 % a.i.) was administered to groups of 24 male and 24 female Sprague-Dawley rats at dietary concentrations of 0, 20, 100, or 500 ppm for two generations (MRID 42248619, 42208406, 422248618). One litter was produced in each generation. Mean premating doses were 1.5, 7.3, and 36.6 mg/kg/day, respectively for F_0 males and 1.7, 8.4, and 42.1 mg/kg/day, respectively for F_0 females. Mean premating doses were 1.9, 9.7, and 47.3 mg/kg/day respectively, for F_1 males and 2.2, 10.6, and 53.6 mg/kg/day,

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respectively, for F_1 females. F_1 adults were chosen from the F_1 pups and weaned onto the same diet as their parents. Animals were given test or control diet for 11 weeks before mating within the same dose group. ~~All animals were continuously exposed to test material either in the diet or during gestation and lactation until sacrifice.~~

There were no deaths or clinical signs of toxicity that were attributable to the presence of fluazinam in the diet. Mean body weight, body weight gain, food consumption and food efficiency among all groups of F_0 males and F_0 females treated with 20 or 100 ppm and F_0 males treated with 500 ppm were similar to the control group means. The F_0 females treated with 500 ppm of the test diet had significantly decreased (82% of control value, $p < 0.001$) overall body weight gain and food consumption (96% of control value, $p < 0.05$) for the premating period. The F_1 males and females treated with 20 or 100 ppm had mean body weights, body weight gains, food consumption, and food efficiencies that were similar to their respective control group means. The F_1 animals treated with 500 ppm had significantly decreased mean body weight gain and food consumption values that were 88% and 92% ($p < 0.001$ and $p < 0.01$) and 85% and 93% ($p < 0.001$ and $p < 0.01$) of the control values for males and females, respectively for the premating period. The decreased body weights continued into gestation for females treated with 500 ppm of both generations; some recovery was made during lactation. The relative liver weights of F_0 and F_1 males and F_0 females treated with 500 ppm were significantly increased compared to the control group. Histopathological findings included an increased incidence of periacinar hepatocytic fatty changes and a decreased incidence of hepatic glycogen pallor among F_0 males treated with 500 ppm compared to the control group. Males in the F_1 generation treated with 100 or 500 ppm also had significantly increased incidences of periacinar hepatocytic fatty changes compared to the control groups. **The NOAEL for parental toxicity is 20 ppm (1.9 mg/kg/day) and the LOAEL is 100 ppm (9.7 mg/kg/day), based on liver pathology (increased incidences of periacinar hepatocytic fatty changes) in F_1 males.**

The fertility index for males and females treated with 500 ppm of the test substance was slightly decreased (n.s.) for F_1 parents compared to the control group. The number of implantation sites observed in F_1 dams was decreased significantly ($p < 0.05$) at 500 ppm (12.2 vs 15.3 in controls) and marginally (n.s.) at 100 ppm (13.1 vs 15.3 in controls). Mean litter size on day 1 was slightly decreased (n.s.) in the 500 ppm groups compared to the control groups in both generations. Mean litter size on day 4 was slightly decreased (n.s.) in the 500 ppm group for F_1 litters, but was significantly decreased ($p<0.05$) in the 500 ppm group for F_2 litters (9.8 ± 3.7 for 500 ppm vs 12.4 ± 3.0 for controls). Pup survival was similar between the treated and control groups for both generations. **The NOAEL for reproductive toxicity is 100 ppm (10.6 mg/kg/day) and the LOAEL is 500 ppm (53.6 mg/kg/day), based on a decreased number of implantation sites and decreased litter sizes to day 4 post partum for F_1 females (F_2 litters).**

Mean overall body weight gain during lactation was significantly decreased (10-13%), among pups in the 500 ppm groups in both generations. The most pronounced effect on pup weight gains occurred between lactation days 7-21. Absolute body weights, however, were not significantly decreased compared to the control groups at any time point during lactation. A slightly decreased developmental time for pinna unfolding, hair growth and eye opening,

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particularly in the F_2 pups, was observed. **The NOAEL for developmental toxicity is 100 ppm (8.4 mg/kg/day) and the LOAEL is 500 ppm (42.1 mg/kg/day), based on decreased body weight gain during lactation for both F_1 and F_2 pups.**

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a 2-generation reproduction study [OPPTS 870.3800 (§83-4)] in rats. No major deficiencies were noted in this study.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: B1216 (Fluazinam Technical)

   Description: pale yellow crystalline powder
   Lot No.: 8412-20
   Purity: 95.3 % a.i.
   Stability of compound: stable under study conditions
   Structure: not given

2. Vehicle and/or positive control

   Labsure Laboratory Animal Diet No. 2 was the vehicle for delivery of the test substance in the diet.

3. Test animals

   Species: Rat
   Strain: CD Sprague-Dawley
   Age and weight at start of study: 4 weeks; males: 75-95 g; females: 60-80 g
   Source: Charles River U.K. Limited, Margate, Kent, England
   Housing: Animals were housed 4/cage (except during mating, after littering and during lactation) in high density propylene cages with wire mesh tops and wood bedding material.
   Diet: Labsure Laboratory Animal Diet No. 2 was available *ad libitum*.
   Water: Tap water was available *ad libitum*.
   Environmental conditions:
   Temperature: 21 °C (ranging from 18-25 °C)
   Humidity: 40 - 70%
   Air Changes: about 15/hour
   Photoperiod: 12 hour light/12 hour dark
   Acclimation period: 13 days

B. PROCEDURES AND STUDY DESIGN

1. In life dates

Start: September 30, 1985; end: June 28, 1986

2. Mating procedure

For mating, each female was paired with a single male from the same dose group. Each morning following pairing, the trays beneath mating cages were examined for the presence of ejected copulation plugs and females were examined for the presence of sperm in a vaginal smear. Once mating had occurred, the pairs were separated and vaginal smearing continued. Females which failed to mate within seven days following pairing were removed and placed with another male from the same treatment group; this could be repeated on one further occasion, thus allowing each animal a maximum of 21 days in which to achieve mating. Sibling pairing was avoided. Day 0 of gestation was designated as the day positive evidence of mating was observed. Day 0 of lactation was the day on which delivery of pups was completed and all viable pups had suckled.

3. Study schedule

F_0 males and females were administered test or control diet for 11 weeks prior to mating. After weaning of the F_1 litters, F_1 generation adults were chosen from the F_1 litters. Selected F_1 animals were given test diets for 11 weeks and then mated to produce the F_2 generation. Dosing for males continued throughout the mating period and until the day before necropsy. Dosing continued for the females during mating, gestation, and lactation of their litters.

4. Animal assignment

F_0 parental animals were assigned to groups based on a computer-generated randomization schedule with stratification by body weights. For the F_1 generation, one male and one female pup were randomly selected from as many F_1 litters as possible. Where less than 24 litters were available, additional animals were selected on a random basis with a maximum of one additional animal per litter. Animal assignment is given in Table 1.

5. Dose selection rationale

Two range-finding studies were conducted to determine appropriate dose levels for this study. In the first study (MRID 42208406), sexually mature male and female Charles River Sprague-Dawley rats were administered fluazinam in the diet at levels of 0, 5, 20, 100, or 1000 ppm for 29 days before mating. Treatment was continued

TABLE 1. Animal assignment

Dose Group	Dietary Conc. (ppm)	*Daily Intake (mg/kg/day)				No. of Parental Animals per Group			
		F_0 Generation		F_1 Generation		F_0 Generation		F_1 Generation	
		Male	Female	Male	Female	Male	Female	Male	Female
0 (Control)	0	0	0	0	0	24	24	24	24
1 (Low)	20	1.5	1.7	1.9	2.2	24	24	24	24
2 (Mid)	100	7.3	8.4	9.7	10.6	24	24	24	24
3 (High)	500	36.6	42.1	47.3	53.6	24	24	24	24

Data taken from Tables 8-9 and 32-33 on pp. 74-75 and 105-106, MRID 42248619
* Mean daily intake mg/kg/day calculated by reviewer.

throughout mating, gestation, and lactation to Day 21 post partum. Body weight gains and food consumption were significantly reduced among females that received 1000 ppm. The number of implantation sites and subsequent litter size were significantly reduced at 1000 ppm. However, post-implantation survival and offspring survival as well as body weight gains were similar among all groups. A decrease in the proportion of female offspring was observed at 1000 ppm. A dose-related increase in relative liver weights was observed among males and females that received 100 and 1000 ppm. Histological examination revealed changes in the liver including periacinar hepatocytic basophilia in adult females treated with 100 ppm and in males and females treated at 1000 ppm and increased periacinar fatty vacuolation in adult males treated at 1000 ppm. From this study it was concluded that the highest dietary concentration to be used in the main multi-generation study should be more than 100 ppm but less than 1000 ppm. The second range-finding study (MRID 42248618) also used sexually mature male and female Charles River Sprague-Dawley rats which were administered fluazinam in the diet at levels of 0, 20, 100, 250, or 500 ppm for 85 days before mating. Treatment was continued throughout mating, gestation, and lactation to Day 21 post partum. Body weight gain of males and females administered 500 ppm was reduced compared to the control group before mating. During lactation and gestation, no clear effect was detected. Food intake was largely unaffected by treatment. Fertility was marginally reduced at 500 ppm, but gestation length, litter size, survival, and offspring body weights and body weight gains were similar to controls. Among adults receiving 250 or 500 ppm, absolute and relative liver weights were increased compared to controls. Females treated with 500 ppm also had absolute and relative kidney weights that were increased compared to the control group. No histopathologically significant observations were made. From this study it was concluded that levels up to 500 ppm of fluazinam in the diet should be suitable for a multi-generation study in the rat.

6. Diet preparation and analysis

Batches of the test diet were prepared weekly during the study. The test material was mixed with a small quantity of the diet and milled in an Ultracentrifugal mill with a 2 mm screen before preparation of the pre-mix. The pre-mix was prepared in a small planetary mixer (Kenwood or Hobart N50), and further dilutions were made with the diet in a horizontal screw-type mixer (Gardner, Type 50L 28GM, maximum capacity 50 kg). Test diets were stored until use in sealed polythene bags at 4 °C for 10-14 days prior to administration. At each preparation, 100 g aliquots of each test diet were saved in sealed aluminium foil sachets and stored at a temperature of approximately 4 °C pending possible future analysis. At the end of each week, any unused diets were destroyed. The homogeneity of the mixing process and the stability of the test material in the diets were determined by analysis of trial preparations before the start of treatment. The achieved concentrations in the test diets were analyzed in duplicate samples taken during the first week and every 4 weeks thereafter.

Results –
Homogeneity analysis: Samples taken and analyzed before initiation of the study indicated mean achieved concentrations from 6 samples for the 20 and 500 ppm diets were 19.7 and 501 ppm, respectively. Homogeneity of the mixing process was not apparent from the description of protocol (i.e. no mention of top, middle, and bottom collection of samples) and results of the analyses presented in Addendum 2.

Stability analysis: After storage at 4 °C for 0, 1, 2, or 3 weeks, the concentrations of test article in the 20 and 500 ppm diets were 19.7, 16.4, 17.1, and 18.7 ppm, and 501, 505, 467, and 453, respectively. After storage at 21 °C for 0, 1, 2, or 3 weeks, the concentrations of test article in the 20 and 500 ppm diets were 19.7, 16.4, 19.9, and 19.2 ppm and 501, 447, 440, and 439 ppm, respectivley.

Concentration analysis: Mean concentrations of the 20, 100, and 500 ppm diets were required by the Sponsor to be within a 10% variation from the target concentration. On three occasions the diets were below the acceptable range and on one of these occasions, fresh diet was prepared and used. For the other two occasions, the diets were within normal limits obtained by the laboratory analysis so they were used.

Results of the dietary analyses show that the test article was stable, and that the actual dosages to the animals were within an acceptable range.

C. OBSERVATIONS

1. Parental animals

All animals were observed once daily for clinical signs and mortality. Body weights for males were determined weekly. Females were weighed weekly until mating was detected, on Days 0, 6, 13, and 20 post coitum and on Days 1, 4, 7, 14, and 21 post partum. Food consumption was recorded weekly until the animals were paired for mating. Food efficiency was calculated.

2. Litter observations

Litter observations were made as shown in Table 2. All females were allowed to litter naturally. All offspring were examined and individually toe-marked on Day 1 post partum (approx. 24 hrs after birth). Pups were weighed on lactation days 0, 4, 7, 14, and 21. On lactation day 4, litters were randomly culled to 4 pups per sex, where possible. All culled pups were necropsied. Offspring were sexed on Days 1, 4 (before and after culling), 7, 14, and 21. Pups were weaned on lactation day 21.

Progression of physical development was assessed on a litter basis by recording the onset and completion of the following parameters.

1) Pinna unfolding: detachment of the edge of the pinna
2) Hair growth: macroscopic observation of generalized growth of body hair
3) Tooth eruption: eruption of upper incisors through the gums
4) Eye opening: separation of the upper and lower eyelids

TABLE 2. Litter observations

Observation	Lactation day				
	Day 1	Day 4	Day 7	Day 14	Day 21
Dead/moribund pups	Daily				
No. pups	X				
Pup weight	X	X	X	X	X
Sex of each pup	X	X[a]	X	X	X
Clinical signs	Daily				

[a]Pre- and post-cull.

3. Postmortem observations

1) Parental animals: Males and females were sacrificed after their respective litters had been weaned and the decision was made by the sponsor that no additional

litter was required. Females that littered but whose litters died before weaning, females that mated but did not give birth, and females that failed to mate were also sacrificed at the same time. All animals were killed by carbon dioxide inhalation. A gross necropsy was performed on all surviving adults and on adults that died or were sacrificed moribund during the study. Tissues from the following (X) organs were dissected and preserved in 4% formaldehyde in buffered saline, with the exception of the testes which were stored in Bouin's fixative. The (XX) tissues were also weighed. Tissues from the control and 500 ppm animals were dehydrated and embedded in paraffin wax, sectioned and stained with haematoxylin and eosin and examined microscopically.

XX	Testes	X	Gross lesions
XX	Epididymides	X	Mammary glands (females with a total litter loss)
XX	Prostate		
XX	Seminal vesicles	X	Pituitary (animals of suspect fertility)
XX	Ovaries	XX	Liver
XX	Uterus		
X	Vagina		

2) Offspring: F_1 offspring not selected for continuation of the study and all F_2 pups were killed at or shortly after weaning. Pups were subjected to gross necropsy and examined internally and externally for macroscopic abnormalities. Specimens of abnormal tissues were retained.

D. DATA ANALYSIS

1. Statistical analyses: Parametric data were compared to the control data using the independent t-test. Non-parametric data were analyzed by the Mann-Whitney U-test.

2. Indices:

Reproductive indices: The following reproductive indices were calculated.

Male/Female Mating index (%) = (No. animals mated/No. animals paired) × 100

Fertility index (%) = (No. animals pregnant/No. animals paired) × 100

Conception rate index (%) = (No. animals achieving pregnancy/No. animals mated) × 100

Gestation index (%) = (No. live litters born/No. confirmed pregnant females) × 100

Estrous cycles - The percentage of females showing regular or other types of estrous cycles before pairing was noted.

Pre-coital interval for females - The time elapsing between initial pairing and detection of mating was noted.

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Offspring viability indices: The following litter indices were calculated.

Live Birth index (%) = (No. pups born alive/No. pups born) × 100

Viability index (%) = (No. pups alive day 4 precull/No. pups born alive) × 100

Lactation index (%) = (No. pups alive day 21/No. pups alive day 4 postcull) × 100

Post implantation survival index (%) = (Total no. of live offspring at Day 1 post partum/No. of implantation sites) x 100

Gestation length was calculated as the number of gestation days up to and including the day on which offspring were first observed. The day of mating was considered Day 1 and in instances where parturition was observed overnight, this value was adjusted by subtracting half a day.

3. Historical control data from 59 studies were included for comparison with concurrent controls.

II. RESULTS

A. PARENTAL ANIMALS

1. Mortality and clinical signs

Signs of a mild transient infection was observed among the majority of F_0 males during weeks 8-9 of treatment. Slight swelling of the throat region, red-rimmed and/or discharging eyes, and nasal discharge were observed generally for about 7 days or less among these animals. One female in group 4 trapped its teeth in the cage lid and was subsequently sacrificed as a humane measure. All remaining F_0 and F_1 parental animals survived to scheduled sacrifice. No treatment-related clinical signs of toxicity were observed in males or females of either generation at any time during the study.

2. Body weight and food consumption

a. Premating - Body weight and food consumption data for the F_0 males are given in Table 3. Absolute body weights and body weight gains of the treated groups were similar to the control group throughout the study. Food consumption and food conversion efficiency by the treated groups was not different from the control group level during the premating interval.

Body weight and food consumption data for the F_0 females during the premating period are given in Table 4. Absolute body weights of the 20 ppm and 100 ppm groups were similar to the controls throughout the premating period. The 500 ppm group had slightly decreased absolute body weights toward the end of the premating period, but statistical significance was not attained. High-dose females

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had significantly reduced body weight gain (82% of control) for the pre-mating period ($p< 0.001$). Low- and mid- dose groups had body weight gain that was similar to the control group. High-dose females had significantly ($p \leq 0.05$) reduced overall food consumption compared to the control group (96% of control). Food consumption by the other treated groups was similar to the control group during the premating interval. Food conversion efficiency seemed to be slightly decreased for the high-dose females during weeks 10-11 compared to control group, although no statistical analysis was performed. The other treatment groups were similar to the control group.

TABLE 3. F_0 males: Mean body weights and food consumption during the premating period when exposed to Fluazinam Technical.

Week of study	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
Body weight (g)				
0	189 ± 9	187 ± 13	188 ± 13	187 ± 14
3	351 ± 22	346 ± 21	348 ± 29	343 ± 22
6	444 ± 34	444 ± 30	446 ± 36	440 ± 30
11 (end of premating)	535 ± 47	539 ± 41	537 ± 46	530 ± 41
14	565 ± 55	561 ± 42	566 ± 48	556 ± 46
18	614 ± 60	620 ± 52	606 ± 49	601 ± 55
Overall weight gain[a]	425	433	418	414
Food consumption prior to mating (g/rat/week)				
1	182 ± 6	182 ± 9	183 ± 10	179 ± 4
3	199 ± 4	205 ± 11	196 ± 10	193 ± 5
7	195 ± 8	197 ± 6	191 ± 7	195 ± 7
11	185 ± 10	195 ± 6	193 ± 4	191 ± 5

Data taken from Tables 2 and 4 on pp. 68 and 70, respectively, MRID 42248619.
[a]Calculated by reviewer.

TABLE 4. F_0 females: Mean body weights and food consumption during the premating period when exposed to Fluazinam Technical.

Week of study	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
Body weight (g)				
0	146 ± 12	147 ± 12	147 ± 9	146 ± 9
3	214 ± 18	213 ± 18	216 ± 14	207 ± 16
6	253 ± 23	253 ± 21	257 ± 20	241 ± 19
9	277 ± 25	278 ± 24	280 ± 22	263 ± 21
11 (end of premating)	298 ± 32	291 ± 26	290 ± 24	270 ± 23
Overall weight gain premating	152	144	143	124 (82) ***
Food consumption (g/rat/week)				
1	128 ± 7	132 ± 10	135 ± 8	131 ± 5
6	138 ± 8	140 ± 7	144 ± 17	133 ± 4
11	129 ± 5	143 ± 5	135 ± 9	126 ± 2
Overall food consumption[b]	1495	1524	1515	1441 (96)*

Data taken from Tables 3 and 5, pp. 69 and 71, respectively, MRID 42248619.
[a]Number in parentheses is percent of control; calculated by reviewer.
[b] Calculated by reviewer.
Significantly different from control: *p ≤ 0.05, ***p ≤ 0.001

Body weight and food consumption data for the F_1 males are given in Table 5. No treatment-related effects on absolute body weights were noted for the 20 or 100 ppm groups. Males in the 500-ppm group had significantly ($p \leq 0.001$) decreased body weight gain for the entire study (88% of control). Overall food consumption was significantly ($p<0.01$) less than the controls for those animals fed 500 ppm (92% of control); other treatment groups remained similar to control throughout the study. Food conversion efficiency was comparable among treatment groups and compared to controls throughout the study; no statistical comparison was conducted to confirm these similarities.

Body weight and food consumption data for the F_1 females during the premating period are given in Table 6. Overall mean body weight gain was significantly decreased ($p< 0.001$) in females fed 500 ppm during the premating period (85% of control). Other groups had mean body weight gains that were similar to the control group. The overall food consumption in the high-dose F_1 females was also significantly ($p<0.01$) decreased compared to the control group for the premating interval (93% of control). Other groups were similar to the control throughout this interval. Food conversion efficiency was similar for treated groups compared to the control group during the premating interval.

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TABLE 5. F_1 males: Mean body weights and food consumption during the premating period when exposed to Fluazinam Technical.

Week of Study	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
	Body weight (g)			
0	72 ± 8	73 ± 8	72 ± 9	70 ± 9
3	233 ± 16	235 ± 20	234 ± 18	223 ± 19
7	427 ± 33	422 ± 38	424 ± 36	395 ± 30
11 (end of premating)	522 ± 49	516 ± 49	515 ± 49	476 ± 37
14	566 ± 56	553 ± 55	548 ± 52	503 ± 39
18 (end of study)	625 ± 67	612 ± 61	612 ± 65	558 ± 48(89)***
Overall weight gain	553	539	540	488 (88)***
	Food consumption prior to mating (g/rat/week)			
1	111 ± 10	117 ± 4	118 ± 10	113 ± 10
4	198 ± 6	197 ± 9	197 ± 5	179 ± 9
8	208 ± 8	209 ± 10	208 ± 6	192 ± 14
11	210 ± 13	207 ± 6	206 ± 7	194 ± 12
Overall food consumption	2110	2120	2111	1947 (92)**

Data taken from Tables 26 and 28 on pp. 99 and 101, respectively, MRID 42248619.
[a]Number in parentheses is per cent of control; calculated by reviewer.
Significantly different from control: *p ≤0.05; **p ≤ 0.01; ***p ≤ 0.001.

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TABLE 6. F_1 females: Mean body weights and food consumption during the premating period when exposed to Fluazinam Technical.

Week of Study	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
	Body weight (g)			
0	67 ± 7	65 ± 8	67 ± 9	64 ± 6
3	168 ± 13	164 ± 11	169 ± 11	156 ± 11
6	229 ± 22	225 ± 19	228 ± 17	206 ± 13
9	262 ± 25	262 ± 22	263 ± 22	233 ± 17
11 (end of premating)	286 ± 28	286 ± 25	284 ± 25	251 ± 19 (88)***
Overall weight gain premating	219	221	217	187 (85) ***
	Food consumption (g/rat/week)			
1	104 ± 10	105 ± 6	106 ± 12	100 ± 7
6	147 ± 9	150 ± 11	143 ± 10	134 ± 5
9	146 ± 8	156 ± 10	151 ± 4	139 ± 6
11	146 ± 14	151 ± 15	145 ± 7	137 ± 5
Overall food consumption	1507	1558	1535	1404 (93) **

Data taken from Tables 27 and 29, pp. 100 and 102, respectively, MRID 42248619.
[a]Number in parentheses is percent of control; calculated by reviewer.
Significantly different from control: $*p \le 0.05$; $**p \le 0.01$, $***p \le 0.001$.

b. Gestation and lactation - Body weight data for the F_0 adult females during gestation and lactation are given in Table 7. During gestation and lactation, absolute body weights of females fed 20 and 100 ppm were similar to the control group. During gestation, the absolute body weights of females fed 500 ppm were significantly decreased (93%, $p< 0.05$) compared to the control group on gestation days 6 and 13. During lactation, these animals gained more weight than the controls, but still had slightly decreased body weights at weaning.

Body weight data for the F_1 adult females during gestation and lactation are given in Table 8. Mean absolute body weights of animals receiving 20 ppm were comparable to the controls throughout gestation and lactation. Females receiving 100 ppm had significantly decreased ($p< 0.05$) body weights on days 13 and 20 of gestation, these were only slight decreases, 98 and 96 % of the control, respectively. This group had body weights that were similar to the control group throughout lactation. In the animals fed 500 ppm, significantly decreased body weights (88 % of controls, $p< 0.01$ or $p< 0.001$) were observed on days 0 and 20 of gestation. On the first day of lactation, the mean body weight of this group was also significantly decreased (89% $p< 0.01$) compared to the control group and remained somewhat decreased (n.s.) throughout the lactation period.

TABLE 7. F_0 Females: Selected mean body weights during gestation and lactation of litter F_1 when exposed to Fluazinam Technical.

Observation	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
Mean body weight (g)				
Day 0 of gestation	289 ± 27	294 ± 27	292 ± 24	272 ± 23
Day 6 of gestation	321 ± 29	328 ± 29	322 ± 27	298 ± 26 (93)[a] *
Day 13 of gestation	350 ± 32	356 ± 32	349 ± 27	325 ± 30 (93)*
Day 20 of gestation	416 ± 35	423 ± 36	416 ± 34	388 ± 35
Day 1 of lactation	324 ± 30	324 ± 30	315 ± 24	301 ± 32
Day 21 of lactation	343 ± 29	347 ± 26	340 ± 24	330 ± 25

Data taken from Tables 13 and 15, pp. 79 and 81, respectively, MRID 42248619.
Significantly different from control; $*p \leq 0.05$.
[a] Percent of control; calculated by reviewer.

TABLE 8. F_1 Females: Selected mean body weights during gestation and lactation of litter F_2 when exposed to Fluazinam Technical.

Observation	Treatment Group			
	0 ppm	20 ppm	100 ppm	500 ppm
Mean body weight (g)				
Day 0 of gestation	291 ± 30	288 ± 27	289 ± 26	257 ± 24 (88)[a]***
Day 6 of gestation	316 ± 29	315 ± 29	313 ± 25	278 ± 23
Day 13 of gestation	347 ± 32	346 ± 33	339 ± 26 (98) *	305 ± 21
Day 20 of gestation	414 ± 39	414 ± 41	397 ± 26 (96)*	359 ± 36 (88)**
Day 1 of lactation	314 ± 33	313 ± 34	315 ± 39	281 ± 30 (89)**
Day 21 of lactation	338 ± 21	334 ± 30	329 ± 25	302 ± 26

Data taken from Tables 37 and 39, pp. 110 and 112, respectively, MRID 42248619.
Significantly different from control; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.
[a] Percent of control, calculated by reviewer.

3. Test substance intake

Test substance intake for the F_0 and F_1 adults is given in Table 1. Overall time-weighted average doses for the premating, gestation, and lactation intervals were not calculated by the study author. As expected, test article intake decreased from the beginning of treatment as the animals grew and gained weight.

4. Reproductive function

For F_0 females, estrous cycle length and periodicity were regular among all groups and the majority of animals in all groups mated at the first estrous during the first four days of pairing. For F_1 females, a high proportion in all groups exhibited acyclic vaginal cytology for periods of 10-18 days before mating. There was no evidence of any association with treatment level and the observations were attributed to pseudopregnancy induced by vaginal smearing during the period before pairing. Approximately half of the F_1 females in al groups mated at the first estrous after pairing. The distribution of pre-coital intervals was distorted by the high frequency of pseudopregnancy.

Neither reproductive function tests for sperm measures nor examinations for sexual maturation of the offspring were conducted in this study.

5. Reproductive performance

The reproductive performances of the F_0 and F_1 animals are summarized in Tables 9 and 10, respectively. For F_0 animals, no treatment-related effects on reproductive performance were observed. For F_1 animals, the fertility index for males and females treated with 500 ppm of the test substance was slightly decreased (n.s.) compared to the control groups. This resulted in a decreased number of females becoming pregnant in this generation at 500 ppm (18 compared to 21 for the controls and other dose groups). The number of implantation sites observed in dams was decreased - significantly ($p < 0.05$) at 500 ppm (12.2 vs 15.3 in controls) and marginally (n.s.) at 100 ppm (13.1 vs 15.3 in controls). The number of implantation sites observed for all groups was within the limits of the historical control values.

TABLE 9. Reproductive performance of the F_0 adults after exposure to Fluazinam Technical.

Observation	0 ppm	20 ppm	100 ppm	500 ppm
Production of F_1 generation				
Number males paired/mated	24/24	24/24	24/24	24/24
Number females paired/mated	24/24	24/24	24/24	24/24
Number females pregnant	23	24	23	24
Mean gestation length (days)*	22.6	22.6	22.6	22.9
Male mating index (%)	100	100	100	100
Female mating index (%)	100	100	100	100
Male fertility index (%)	96	100	96	100
Female fertility index (%)	96	100	96	100
Number of Implantation sites	15.0	15.5	16.0	14.3

Data taken from Tables 11 and 14, pp. 77 and 80, respectively, MRID 42248619.
* Calculated by reviewer.

TABLE 10. Reproductive performance of the F_1 adults after exposure to Fluazinam Technical.

Observation	0 ppm	20 ppm	100 ppm	500 ppm
Production of F_2 generation				
Number males paired/mated	23[b] /22	24/23	24/23	24/24
Number females paired/mated	24/24	24/23	24/24	24/24
Number females pregnant	21	21	21	18
Mean gestation length (days)[a]	22.6	22.6	22.9	22.9
Male mating index (%)	96	96	96	100
Female mating index (%)	100	96	100	100
Male fertility index (%)	87	88	83	75
Female fertility index (%)	88	88	88	75
Number of Implantation sites	15.3	15.1	13.1	12.2*

Data taken from Tables 35 and 38, pp. 108 and 111, respectively, MRID 42248619.
[a]Calculated by reviewer.
[b] A mistake was made in the sexing of one male pup, the mistake was noticed when the animal became pregnant
* $p < 0.05$ compared to the control group.

6. Parental postmortem results

   a. Organ weights - Females in the F_0 generation had significantly decreased (93%, $p<0.05$) terminal body weights compared to the control group. Males and females in the F_1 generation had significantly decreased (89%, $p< 0.01$) terminal body weights compared to their respective control groups. The relative liver weights of F_0 males and females and F_1 males treated with 500 ppm were significantly increased ($p< 0.01$) compared to the respective control groups (Table 11). Statistically significant ($p<0.01$) decreases in absolute weights of epididymides and ovaries for F_1 animals at 500 ppm were considered to be the result of decreased body weights of these animals and not a treatment-related effect because the relative organ/body weight ratios for these organs were not affected. Other significant differences in organ weights were sporadic, not dose-related, not consistent between sexes or generations, or due to decreased terminal body weights.

   b. Pathology

      1) Macroscopic pathology - No abnormalities were observed which could be attributed to treatment with the test substance among either sex in either generation of adult animals.

      2) Microscopic pathology - A statistically significant ($p<0.05$) increased incidence of periacinar hepatocytic fatty changes was observed among F_0 males treated with 500 ppm (6/24, 4/24, 10/24 and 14/24* for the control, 20, 100 and 500 ppm groups, respectively). A statistically significant ($p<0.01$) increased incidence of periacinar hepatocytic fatty changes was also observed among F_1 males treated with 100 and 500 ppm (2/23, 4/24, 11/24** and 11/24** for the control, 20, 100 and 500 ppm groups, respectively). A statistically significant ($p<0.001$) decreased incidence of hepatic glycogen pallor was observed among F_0 males treated with 500 ppm (23/24, 18/24, 19/24 and 9/24*** for the control, 20, 100 and 500 ppm groups, respectively).

TABLE 11: Organ weights for male and female rats administered Fluazinam Technical in the diet for two generations

Organ	0 ppm	20 ppm	100 ppm	500 ppm
F_0 Males				
Terminal body wt. (g)	607.1 ± 60.7	610.7 ± 53.0	598.5 ± 49.8	590.0 ± 53.6
Liver				
absolute (g)	22.3	22.1	22.2	23.3
relative (%)	3.67	3.61	3.71	3.95**
F_0 Females				
Terminal body wt. (g)	319.5 ± 27.8	320.7 ± 26.1	316.0 ± 20.7	297.9 ± 23.9 (93)*
Liver				
absolute (g)	13.5	14.3	14.0	14.0
relative (%)	4.22	4.44*	4.43*	4.73**
F_1 Males				
Terminal body wt. (g)	623.3 ± 67.2	607.4 ± 61.8	607.4 ± 64.5	555.3 ± 48.0 (89)**
Liver				
absolute (g)	22.5	21.3	23.1	21.7
relative (%)	3.61	3.51	3.78	3.91**
F_1 Females				
Terminal body wt. (g)	323.5 ± 29.4	323.5 ± 31.4	325.7 ± 30.8	288.2 ± 22.7 (89)**
Liver				
absolute (g)	12.8	13.3	13.0	11.7 *
relative (%)	3.95	4.12	4.0	4.08

Data taken from Tables 23-24 and 47-48, pp. 93-95 and 123-125, MRID 42248619.
Significantly different from control: *p ≤ 0.05; **p ≤ 0.01.

B. OFFSPRING

1. Viability and clinical signs

Viability data for the F_1 and F_2 litters are given in Tables 12 and 13, respectively. Mean litter size on day 1 was slightly decreased (n.s.) in the 500 ppm groups compared to the control groups in both generations. Mean litter size on day 4 was slightly decreased (n.s.) In the 500 ppm group for F_1 litters, but was significantly decreased ($p<0.05$) in the 500 ppm group for F_2 litters (9.8 $\pm$ 3.7 for 500 ppm vs 12.4 $\pm$ 3.0 for controls). Mean litter size on day 1 and day 4 was also slightly decreased (n.s.) In the 100 ppm group for F_2 litters. The number of live births and pup survival were similar between the treated and control groups for both generations.

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TABLE 12. Viability of F_1 litters during lactation and exposure to Fluazinam Technical.

Observation/study time	0 ppm	20 ppm	100 ppm	500 ppm
Number of litters	23	24	23	23
Whole litter losses	0	0	0	0
Sex ratio male:female on day 1	1 : 0.87	1 : 1.15	1 : 1.01	1 : 1.21
Mean litter size on day 1	13.2 ± 3.7	14.2 ± 2.7	14.4 ± 3.0	12.4 ± 3.1
Mean litter size on day 4 (before cull)	13.0 ± 3.1	13.8 ± 2.4	13.6 ± 2.7	11.9 ± 3.3
Live birth index (%)	98	99	94	98
Post-implantation survival index (%)	88	92	91	88
Viability index days 0-4 (%)	94	98	95	87
Lactation index days 14 and 21 (%)	99	100	99	97

Data taken from Tables 16, 17, and 20, pp. 82, 83, and 86, respectively, MRID 42248619.

TABLE 13. Viability of F_2 litters during lactation and exposure to Fluazinam Technical.

Observation/study time	0 ppm	20 ppm	100 ppm	500 ppm
Number of litters	21	21	20	17
Whole litter losses	0	0	1	1
Sex Ratio male:female on Day 1	1 : 0.91	1 : 1.17	1 : 1.13	1 : 1.01
Mean litter size on day 1	13.4 ± 3.1	14.2 ± 2.9	11.9 ± 4.3	11.2 ± 3.4
Mean litter size on day 4 (before cull)	12.4 ± 3.0	12.8 ± 2.9	11.3 ± 3.3	9.8 ± 3.7*
Live birth index (%)	96	99	100	98
Post-implantation survival index (%)	89	93	91	88
Viability index days 0-4 (%)	88	90	85	87
Lactation index days 14 and21 (%)	90	98	96	97

Data taken from Tables 40, 41, and 44, pp. 113, 114 and 117, respectively, MRID 42248619.

2. Offspring development

Offspring developmental time frames for the F_1 and F_2 litters are described in Tables 14 and 15, respectively. Generally, development of F_1 pups was comparable to controls although eye opening was significantly earlier in the F_1 500 ppm treated group compared to the control group. Physical development of F_2 pups tended to occur somewhat earlier in this study compared to the historical controls. For the F_2 pups, pinna unfolding, hair growth, and eye opening occurred significantly ($p < 0.05$ or 0.01) earlier in the 500 ppm litters compared to the control group.

TABLE 14. Development (in days) of F_1 pups during lactation and exposure to Fluazinam Technical.

Observation	0 ppm		20 ppm		100 ppm		500 ppm	
	Onset	Completion	Onset	Completion	Onset	Completion	Onset	Completion
Pinna Unfolding	2.5	3.3	2.5	3.4	2.7	3.5	2.2	3.1
Hair Growth	2.4	3.0	2.4	3.3	2.7	3.3	2.4	3.3
Tooth Eruption	8.8	10.5	9.1	11.2	8.5	10.8	8.5	10.7
Eye Opening	13.8	14.7	13.6	14.7	13.3	14.5	13.0**	13.8**

Data taken from Table 19, p. 85, MRID 42248619.
** Significantly different from control $p< 0.01$

TABLE 15. Development (in days) of F_2 pups during lactation and exposure to Fluazinam Technical.

Observation	0 ppm		20 ppm		100 ppm		500 ppm	
	Onset	Completion	Onset	Completion	Onset	Completion	Onset	Completion
Pinna Unfolding	2.9	3.9	2.9	4.1	2.4	3.4	2.3*	3.2**
Hair Growth	2.8	3.8	2.9	3.8	2.6	3.3	2.2**	3.2**
Tooth Eruption	9.3	10.9	9.3	11.0	9.2	10.9	8.9	10.2
Eye Opening	13.7	14.8	14.0	15.0	13.6	14.7	13.4	14.1**

Data taken from Table 43, p.116, MRID 42248619.
Significantly different from control, *$p< 0.05$; **$p< 0.001$

3. Body weight

Selected body weights and body weight gains of the F_1 pups during lactation are given in Table 16. Absolute body weights and overall body weight gains among pups in the 20 and 100 ppm groups were similar to the control group. Absolute body weights of pups in the 500 ppm were not significantly decreased compared to the control, but were slightly decreased beginning with day 7 of lactation and continuing until weaning. The overall weight gain of pups in the 500 ppm group was significantly decreased (90%, $p< 0.001$) compared to the control during the lactation period which could be attributed to decreased body weight gain between days 7-21.

Selected body weights and body weight gains of the F_2 pups during lactation are given in Table 17. Pup body weights and body weight gain among the 20 and 100 ppm groups were similar to the control group during lactation. Pups in the 500 ppm group had slightly decreased (n.s.) absolute body weight during days 14-21 of the lactation period. The overall body weight gain of pups in the high-dose group was

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significantly decreased (87 %, $p < 0.01$) compared to the control group for the lactation period. This effect was mainly the result of decreased body weight gain between days 14-21.

TABLE 16. Mean body weights (g) of F_1 pups during lactation and exposure to Fluazinam Technical.				
Day of lactation	**0 ppm**	**20 ppm**	**100 ppm**	**500 ppm**
Day 1	6.3 ± 0.8	6.1 ± 0.8	6.2 ± 0.8	6.1 ± 0.7
Day 4 (precull)	9.0 ± 1.4	8.3 ± 1.5	8.3 ± 1.8	8.5 ± 1.4
Day 4 (postcull)	9.0 ± 1.3	8.5 ± 1.5	8.5 ± 1.7	8.6 ± 1.3
Day 7	15.2 ± 1.8	14.4 ± 2.2	14.4 ± 2.3	14.0 ± 2.4
Day 14	32.4 ± 3.0	31.4 ± 3.1	31.7 ± 2.6	29.6 ± 3.6
Day 21	53.5 ± 4.6	51.7 ± 5.4	52.0 ± 4.1	48.4 ± 5.2
Overall weight gain[b]	47.2	45.6	45.8	42.3 (90)[a]***

Data taken from Table 18, p. 84, MRID 42248619.
[a]Number in parentheses is per cent of control.
[b]Calculated by reviewer.
Significantly different from controls; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

TABLE 17. Mean body weights (g) of F_2 pups during lactation and exposure to Fluazinam Technical.				
Day of lactation	**0 ppm**	**20 ppm**	**100 ppm**	**500 ppm**
Day 1	5.8 ± 0.7	5.7 ± 0.6	6.2 ± 0.8	6.2 ± 0.6
Day 4 (precull)	7.7 ± 1.5	7.4 ± 1.3	8.6 ± 1.8	8.1 ± 1.3
Day 4 (postcull)	7.8 ± 1.4	7.6 ± 1.3	8.6 ± 1.8	8.1 ± 1.3
Day 7	12.9 ± 3.1	12.7 ± 2.3	14.2 ± 3.0	12.9 ± 2.3
Day 14	30.5 ± 3.6	28.8 ± 3.5	31.1 ± 4.7	27.6 ± 3.4
Day 21	50.8 ± 5.3	48.3 ± 5.7	51.4 ± 6.4	45.5 ± 4.5
Overall weight gain[b]	45	42.6	45.2	39.3 (87)[a]**

Data taken from Table 42, p. 115, MRID 42248619.
[a]Number in parentheses is per cent of control.
[b]Calculated by reviewer.
Significantly different from controls; *$p \leq 0.05$, **$p \leq 0.01$.

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4. Offspring postmortem results

   a. Organ weights - not reported

   b. Pathology

      1) Macroscopic pathology - Among pups of both generations that died before weaning, the only remarkable finding was the absence of milk/food in their stomachs. No treatment-related lesions were found in pups at gross necropsy.

      2) Microscopic pathology - No treatment-related histological lesions were observed in either generation.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The study authors concluded that administration of fluazinam in the diet of male and female rats throughout two successive generations at the lowest level of 20 ppm did not produce adverse effects upon somatic growth or reproductive performance. At the mid-dose of 100 ppm, the authors felt that slight reductions in bodyweight gain of F_1 females during gestation, in the number of F_2 implantation sites, and litter sizes up to Day 4 post partum were adverse effects attributable to administration of the test substance.

At 500 ppm, F_0 females had reduced body weight gains compared to the control during maturation and gestation. F_1 males and females had decreased overall body weight gains for the maturation and gestation periods. Food consumption was significantly decreased among F_0 females and among F_1 males and females during the premating period. At 500 ppm, pup body weight gains during lactation were reduced in both generations. The reproductive function of F_0 adults was unaffected by the administration of B1216. For F_1 adult females, the fertility index was decreased. The number of F_2 implantation sites was decreased and litter size was reduced. No other effects on reproductive parameters were noted.

Increased relative liver weight was observed among F_0 males and females and F_1 males at 500 ppm. Histopathological evaluation failed to reveal changes that were considered toxicologically relevant.

Therefore, the authors felt that a NOEL of 20 ppm was appropriate for the effects of the test substance on somatic growth and development, and 100 ppm was considered to be an appropriate NOEL for effects on reproduction.

B. REVIEWER'S DISCUSSION

1. Parental Toxicity

There were no deaths attributable to the administration of fluazinam in this study. No treatment-related clinical signs of toxicity were observed in parental animals of either generation at any time during the study.

Body weight gain was significantly reduced among F_0 and F_1 females and F_1 males in the 500 ppm groups throughout the maturation and gestation periods. Food consumption was significantly decreased for females in both the F_0 and F_1 and for males in the F_1 generation at the high-dose (500 ppm). Slightly decreased body weight gain was also observed in F_1 females during gestation at 100 ppm. These females partially recovered from the body weight deficit during the lactation period.

High-dose adult females in the F_0 generation and adult males and females in the F_1 generation had decreased terminal body weights at sacrifice. Increased relative liver weights were also observed among F_0 and F_1 males and F_0 females treated at the high-dose. Histopathological changes in the livers of adult F_0 and F_1 males (increased periacinar hepatocytic fatty changes and decreased glycogen pallor) treated with 500 ppm were observed. Increased periacinar hepatocytic fatty changes were also observed in adult F_1 males at 100 ppm.

The NOAEL for parental toxicity is 20 ppm and the LOAEL is 100 ppm, based on liver pathology (increased periacinar hepatocytic fatty changes) in adult F_1 males.

2. Reproductive toxicity

The slightly decreased fertility index for F_1 animals at 500 ppm is considered to be an equivocal effect of the test material. The decreased number of implantation sites and decreased litter size for the 500 ppm F_1 parents (F_2 litters) is considered to be treatment-related. At 100 ppm, however, the same effects did not achieve statistical significance and were within the limits of the historical controls.

The NOAEL for reproductive toxicity is 100 ppm and the LOAEL is 500 ppm, based on a decreased number of implantation sites and decreased litter sizes to day 4 post partum for F_1 parents (F_2 litters).

3. Developmental toxicity

Mean overall body weight gain during lactation was significantly decreased (10-13%), among pups in the 500 ppm groups in both generations. The most pronounced effect on pup weight gains occurred between lactation days 7-21 and continued into the premating period of the F_1 generation. Absolute body weights, however, were not significantly decreased compared to the control groups at any time point during lactation. The decreased developmental time for pinna unfolding, hair growth and eye opening, particularly in the F_2 pups, is considered to be an equivocal effect of the test material.

The NOAEL for developmental toxicity is 100 ppm and the LOAEL is 500 ppm, based on decreased body weight gain during lactation for both F_1 and F_2 pups.

C. STUDY DEFICIENCIES

No major deficiencies were identified in the conduct of this study. A minor deficiency is that adequate testing for homogeneity of the mixing process for the test diets was not conducted (from the reported protocol) using samples from the top, middle, and bottom of the mixer. Also, the study author did not calculate time-weighted average doses of the test article for the premating period, and food consumption was not reported for the females during gestation and lactation.

D. CORE CLASSIFICATION

This study is classified as **Acceptable/Guideline** and satisfies the requirements for a reproduction study [OPPTS 870.3800 (§83-4)] in rats.

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