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

Edwin Budd, Date 2/16/01

014613

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

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity feeding study - Mouse [OPPTS 870.4200 (§83-2b)].

DP BARCODE: D258235
P.C. CODE: 129098

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

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

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

CITATION: Mayfield, R., S. Burton, D. Crook et al. (1988) Fluazinam technical (B-1216): potential carcinogenicity study in dietary administration to mice for 104 weeks. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 9/87264, September 29, 1988. MRID 42208405. Unpublished.

Mayfield, R. (1996) Amendment and addendum to report no ISK 9/87264: technical fluazinam potential carcinogenicity to mice (MRID 42208405). Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 9/87264, December 19, 1996. MRID 44807220. Unpublished.

Amyes, S.J., S.M. Macrae, J.C. Whitney (1983) B-1216: Four-week toxicity study in mice. Life Science Research, Stock, Essex, CM4 9PE, England, Document Nos. 82/ISK036/067 and 82/ISK036/215, June 9, 1983. MRID 44807212. Unpublished.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 carcinogenicity study (MRID 42208405, 44807220, 44807212), Fluazinam (95.3% a.i., lot no. 8412-20) was administered to groups of 52 male and 52 female CD®-1 mice in the diet at concentrations of 0, 0, 1, 10, 100, or 1000 ppm. There were 2 control groups. The test diets were given for 104 weeks. These concentrations resulted in mean daily compound intakes of 0.12, 1.1, 10.7, and 107 mg/kg/day for 1 ppm, 10 ppm, 100 ppm, and 1000 ppm, respectively, for males and 0.11, 1.2, 11.7, and 117 mg/kg/day, respectively, for females. Additional microscopic review of brain and spinal cord was presented in MRID 44807220. A four-week-range finding study (MRID 44807212) using 0, 10, 50, 250, or 3000 ppm in the diet was also conducted.

Treatment with Fluazinam did not result in treatment-related changes in survival, clinical signs, body weights, body weight gains, food consumption or hematology parameters. The group mean liver weights adjusted for body weight were increased in males and females by 45% and 30%, respectively, at 1000 ppm compared to the controls, and by 15% in females at 100 ppm after 104 weeks of treatment ($p<0.01$). Microscopic examination showed increased incidences of liver areas containing basophilic hepatocytes (controls, 12%; 1000 ppm, 38%, $p<0.01$) and/or eosinophilic vacuolated hepatocytes (controls, 1%; 100 ppm, 8%, $p<0.05$; 1000 ppm 19%, $p<0.01$) in treated males compared to the controls. Increased incidences of granulomatous hepatitis of minimal severity were seen in high-dose males (controls, 11%; 1000 ppm, 37%, $p<0.01$) and females (controls, 11%; 1000 ppm, 21%, $p<0.01$). Higher incidences of aggregates of brown pigmented macrophages were seen in the livers of treated males (controls, 13%; 100 ppm, 27%, $p<0.05$; 1000 ppm, 19%, $p<0.01$) and females (controls, 15%; 1 ppm, 40%, $p<0.01$; 10 ppm, 21%, NS; 100 ppm, 38%; 1000 ppm, 50%, $p<0.01$). Granulomatous hepatitis and brown pigmented macrophage aggregates were most commonly seen in mice that survived until the end of the study. The only effects that were not associated with the liver were an increased incidence of thymic hyperplasia in high-dose females (controls, 5%; 1000 ppm, 21%, $p <0.01$), and increased incidences of cystic thyroid follicles in high-dose males (controls, 23%; 1000 ppm, 52%, $p<0.01$) and high-dose females (controls, 16%; 1000 ppm, 33%, $p<0.01$).

The central nervous systems of the animals were re-examined and the results reported in an addendum to the main study (MRID 44807220). Treatment-related increases in the incidences and severity of vacuolation of white matter occurred in the brains of males at 1000 ppm and increased severity of white matter vacuolation was seen in the brains of females at 1000 ppm compared to the control groups. No clear effect of treatment on the incidence or severity of white matter vacuolation in the spinal cord was seen in either sex, and no treatment-related effects were seen at 1, 10, or 100 ppm.

The LOAEL is 100 ppm in the diet (10.7 mg/kg/day for males; 11.7 mg/kg/day for females), based on increased incidences of brown pigmented macrophages in the liver of both sexes, increased incidences of eosinophilic vacuolated hepatocytes in males, and increased liver weights in females. The NOAEL was 10 ppm (1.1 mg/kg/day for males; 1.2 mg/kg/day for females).

In this study, there were statistically significant positive trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas for the male mice. There were also statistically significant increases by pair-wise comparison of the male high dose group (1000 ppm) with the controls for hepatocellular adenomas (34% vs 16% in controls), for hepatocellular carcinomas

(34% vs 19% in controls) and for combined hepatocellular adenomas/carcinomas (62% vs 33% in controls). The incidence of hepatocellular adenomas (34%) at the highest dose level for males exceeded the highest incidence in the historical control data for 1981-1983 (4-27%) and for 1986-1988 (8-23%), and the incidence of hepatocellular carcinomas (34%) for the highest dose level for males exceeded the highest incidence in the historical control data for 1986-1988 (5-13%), but not for 1981-1983 (12-38%). There were no treatment-related tumors observed in the female mice in this study. The highest dose level tested was considered to be adequate but not excessive because liver and brain toxicity were observed in the male and female mice at 1000 ppm. Although there were no significant changes in survival or body weight gain, mean liver weight gains were increased in males and females and histopathological changes were observed in the livers and brain (vacuolation of the white matter) of males and females.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200 (83-2b)] in mice. An additional study has been done following this study with higher concentrations of fluazinam (see MRID 44807222).

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

Attachment #1

Original DER prepared for this study by Oak Ridge Natioinal Laboratory

DATA EVALUATION REPORT

FLUAZINAM TECHNICAL

STUDY TYPE: ONCOGENICITY FEEDING – MOUSE [OPPTS 870.4200 (83-2b)]
MRID 42208405–44807212

Prepared for

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

Chemical Hazard Evaluation Group
Toxicology and Risk Analysis Section
Life Sciences Division
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Oak Ridge, TN 37830
Task Order No. 99-51F, M

Primary Reviewer: A. A. Francis, M.S., D.A.B.T.	Signature: Date:	Robert N. Ross for A.A. Francis DEC 2 0 1999
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Disclaimer

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

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EPA Reviewer: E. Budd, M.S. Edwin R. Budd, Date 3/21/00
Registration Action Branch 2 (7509C)
EPA Work Assignment Manager: M. Copley, D.V.M., D.A.B.T. M Copley, Date 9/26/00
Registration Action Branch 1 (7509C)

DATA EVALUATION RECORD

STUDY TYPE: Oncogenicity Feeding - Mouse [OPPTS 870.4200 (§83-2b)].

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

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

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

CITATION: Mayfield, R., S. Burton, D. Crook et al. (1988) Fluazinam technical (B-1216): potential carcinogenicity study in dietary administration to mice for 104 weeks. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 9/87264, September 29, 1988. MRID 42208405. Unpublished.

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Amyes, S.J., S.M. Macrae, J.C. Whitney (1983) B-1216: Four-week toxicity study in mice. Life Science Research, Stock, Essex, CM4 9PE, England, Document Nos. 82/ISK036/067 and 82/ISK036/215, June 9, 1983. MRID 44807212. 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 an oncogenicity study (MRID 42208405, 4807220, 44807212), Fluazinam (95.3% a.i., lot no. 8412-20) was administered to groups of 52 male and 52 female CD®-1 mice in the diet at concentrations of 0, 0, 1, 10, 100, or 1000 ppm. There were 2 control groups. The test diets were given for 104 weeks. These concentrations resulted in mean daily compound intakes of 0.12, 1.12, 10.72, and 107 mg/kg/day for 1 ppm, 10 ppm, 100 ppm, and

1000 ppm, respectively, for males and 0.11, 1.16, 11.72, and 117 mg/kg/day, respectively, for females. Additional microscopic review of brain and spinal cord was presented in MRID 44807220. A four-week-range finding study (MRID 44807212) using 0, 10, 50, 250, or 3000 ppm in the diet was also conducted.

Treatment with Fluazinam did not result in treatment-related changes in survival, clinical signs, body weights, body weight gains, food consumption or hematology parameters. The group mean liver weights adjusted for body weight were increased in males and females by 45% and 30%, respectively, at 1000 ppm compared to the controls, and by 15% in females at 100 ppm after 104 weeks of treatment ($p<0.01$). Microscopic examination showed increased incidences of liver areas containing basophilic hepatocytes (controls, 12%; 1000 ppm, 38%, $p<0.01$) and/or eosinophilic vacuolated hepatocytes (controls, 1%; 100 ppm, 8%, $p<0.05$; 1000 ppm 19%, $p<0.01$) in treated males compared to the controls. Increased incidences of granulomatous hepatitis of minimal severity were seen in high-dose males (controls, 11%; 1000 ppm, 37%, $p<0.01$) and females (controls, 11%; 1000 ppm, 21%, $p<0.01$). Higher incidences of aggregates of brown pigmented macrophages were seen in the livers of treated males (controls, 13%; 100 ppm, 27%, $p<0.05$; 1000 ppm, 19%, $p<0.01$) and females (controls, 15%; 1 ppm, 40%, $p<0.01$; 10 ppm, 21%, NS; 100 ppm, 38%; 1000 ppm, 50%, $p<0.01$). Granulomatous hepatitis and brown pigmented macrophage aggregates were most commonly seen in mice that survived until the end of the study. The only effects that were not associated with the liver were an increased incidence of thymic hyperplasia in high-dose females (controls, 5%; 1000 ppm, 21%, $p <0.01$), and increased incidences of cystic thyroid follicles in high-dose males (controls, 23%; 1000 ppm, 52%, $p<0.01$) and high-dose females (controls, 16%; 1000 ppm, 33%, $p<0.01$).

The central nervous systems of the animals were re-examined and the results reported in an addendum to the main study (MRID 44807220). Treatment-related increases in the incidences and severity of vacuolation of white matter occurred in the brains of males at 1000 ppm and increased severity of white matter vacuolation was seen in the brains of females at 1000 ppm compared to the control groups. No clear effect of treatment on the incidence or severity of white matter vacuolation in the spinal cord was seen in either sex, and no treatment-related effects were seen at 1, 10, or 100 ppm.

The LOAEL is 100 ppm in the diet (10.72 mg/kg/day for males; 11.72 mg/kg/day for females), based on increased incidences of brown pigmented macrophages in the liver of both sexes, increased incidences of eosinophilic vacuolated hepatocytes in males, and increased liver weights in females. The NOAEL was 10 ppm (1.12 mg/kg/day for males; 1.16 mg/kg/day for females).

Treatment of CD®-1 mice for up to 104 weeks resulted in a significant increase in the hepatocellular adenoma incidence in high-dose males under the conditions of this study (controls, 14%; 1000 ppm 33%, $p<0.05$). The incidence of hepatocellular carcinoma was also increased in high-dose males, but the increase was not statistically significant by the tests applied by the study authors (controls, 17%; 1000 ppm, 33%, NS). No increases in hepatocellular tumor incidences were seen in treated females compared to the controls. Historical control data supplied with the study showed the hepatocellular adenoma incidence in males ranged from

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about 4% to 27% in mouse studies of similar duration, and the hepatocellular carcinoma incidence ranged from 12% to 38%. The incidence for hepatocellular adenomas for high-dose males in this study (33%) slightly exceeded the upper range of historic controls (27%). The dosing was adequate for an oncogenicity study based on the liver and brain toxicity at 1000 ppm.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200 (83-2b)] in mice. An additional study has been done following this study with higher concentrations of fluazinam (see MRID 44807222).

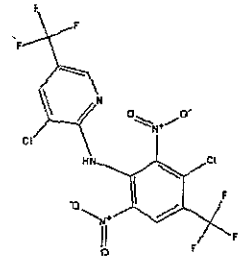
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: B-1216 (Fluazinam)

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



2. Vehicle and/or positive control: The test material was mixed with feed; a positive control was not included in this study.

3. Test animals: Species: mouse

   Strain: CD®-1
   Age and weight at study initiation: age: approximately 44 days; weight (group means at week 0): males, 29 - 30 g; females, 23 g
   Source: Charles River Breeding Laboratories, Manston, Kent, U.K.

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Housing: animals were segregated according to sex and housed 4/cage in solid bottom polypropylene cages with sterile, sifted sawdust bedding (for duration of the study).
Diet: Scientific Feeds/ Labsure Laboratory Animal Diet No. 2, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 21°C
Relative humidity: 50%
Ventilation: not reported
Light cycle: 12 hours light: 12 hours dark
Acclimation period: 17 days

B. STUDY DESIGN

1. In life dates

Start: February 4, 1985; end: February 18, 1987

2. Animal assignment

Animals were stratified by body weight and randomly assigned to the test groups listed in Table 1.

TABLE 1. Study design

Test group	Dietary concentration (ppm)	Dose to animals[a] (mg/kg/day)		Number of animals	
		Male	Female	Male	Female
Control 1	0	0	0	52	52
Control 2	0	0	0	52	52
3	1	0.12	0.11	52	52
4	10	1.12	1.16	52	52
5	100	10.72	11.72	52	52
6	1000	107	117	52	52

Data taken from pp. 27 and 38, MRID 42208405.
[a]Daily dietary Fluazinam consumption was calculated from the group mean food consumption and body weight data, and was based on nominal dietary levels of Fluazinam assuming 95.3% purity.

3. Dose selection

The dietary concentrations selected for this study were based on an earlier 4-week study (document nos. 82/ISK036/067 and 82/ISK036/215, MRID 44807212). Groups of 10 male and 10 female CD®1 mice were given concentrations of 0, 10, 50, 250, or

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3000 ppm fluazinam (B-1216) in the diet for 4 weeks. There were no significant changes in body weights between treated and control animals; however, group mean body weight gain was slightly less in both sexes at 250 and 3000 ppm. The body weight change did not show a clear dose dependency especially in females. Platelet counts were marginally elevated in males at 3000 ppm and total blood cholesterol was slightly higher in both sexes at 3000 ppm compared to the controls. Phospholipid concentration was slightly increased in females and marginally increased in males at 3000 ppm. Blood glucose concentrations were increased in females at 250 and 3000 ppm compared to the control group. Absolute and relative (to body weight) liver weights were increased at 3000 ppm in both sexes and the absolute and relative kidney weights were increased in females at 3000 ppm. Hepatocyte periacinar hypertrophy incidences and severity were increased in both sexes at 3000 ppm compared to the control groups. A more detailed outline of the 4-week study is included in Appendix 1.

4. Diet preparation and analysis

A pre-mix was prepared by adding the ground fluazinam to the Scientific Feeds/Labsure Laboratory Animal Diet No. 2 and mixing in an inflated polythene bag for at least 3 minutes. The dietary concentrations used in the study were prepared by diluting the pre-mix with untreated diet and mixing in a double cone blender for at least 7 minutes. Fresh pre-mix and test diets were prepared weekly and stored at 4°C until used. The mixtures were corrected for purity (95.3%) of the test material. Duplicate samples from the 1 and 1000 ppm mixes were taken from the top, middle, and bottom of the diet mixer to test for homogeneity prior to the beginning of the study. The stability of fluazinam in the 1 and 1000 ppm dietary mixtures was tested after storage for 3 and 5 weeks at ambient temperature, and after storage at -4°C for 2, 3, and 4 weeks. All dietary concentrations were analyzed for fluazinam content at approximately 2-week intervals through week 13 of the study, and at approximately 4-week intervals through week 105. The analyses for the control and 1 ppm groups were repeated or different 1 ppm mixes were analyzed at various times during the study and during week 107.

Results

Homogeneity – The average concentrations of duplicate samples of the test material in the 1 ppm mixture taken from the top, middle and bottom of the container ranged from 93 to 115% of the mean concentration, and the concentrations in the 1000 ppm dietary preparations were ± 4% of the mean. The 1000 ppm mixtures were prepared using 1 pre-mix preparation. The 1 ppm dietary mixture was prepared using either 2 or 3 pre-mix preparations; when 3 pre-mixes were used the concentrations in the dietary samples were 93 to 106% of the mean concentration. The relative standard deviations for the 1 ppm samples prepared using 3 pre-mixes was 2.99%, and the relative standard deviation for the 1000 ppm samples was 1.18%,

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Stability – The analysis of dietary mixtures containing either 1 or 1000 ppm fluazinam stored for up to 4 weeks at +4°C indicated no loss of the test material. The fluazinam content of the 1 ppm mixture after storage at room temperature was 92% and 83% after 3 and 5 weeks, respectively, compared to the initial concentration. The 1000 ppm mixture stored for 3 weeks at ambient temperature was 95% of the week 0 concentration and was about 91% after 5 weeks.

Concentration analysis – The mean concentration analyses showed agreement with variations generally within ±20% of the target concentration for the 1 and 10 ppm dietary mixtures and within ±10% of the target concentrations for the 100 and 1000 ppm dietary concentrations. The only exceptions to these limits included variations of 24, 31, and 23% in the 1 ppm mixture and one variation of 11% in the 1000 ppm mixture.

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

Food consumption, body weight, hematology, and organ weight data were analyzed for heterogeneity of variance between treatment groups utilizing Levene's test. The responses achieved at increasing dose levels were compared with the corresponding controls by William's test. Brain weights of treated animals were compared with the controls using Fisher's exact test, and trends were analyzed with Mantel's test. Lung and liver tumors were analyzed by the time-to tumor method recommended by the International Agency for Research on Cancer. Group comparisons for significant differences and trends in liver cell tumors were done with one-tailed and two-tailed tests. The tests for differences and trends in liver cell tumor incidences were repeated using Fisher's exact test and the Mantel-Haenzel test.

The two control groups were combined as a single group in the statistical analyses, and the sexes were analyzed separately. General linear model analyses were used as appropriate. Statistical significance was flagged at $p<0.05$.

C. METHODS

1. Observations

Animals were inspected twice daily for signs of toxicity and mortality. All animals were given a detailed examination once daily, excluding weekends and holidays, for the first 4 weeks of the study after which the examinations were done once a week.

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2. Body weight

Animals were weighed at weekly intervals from 1 week prior to the study initiation to study termination.

3. Food consumption and compound intake

Food consumption per cage was determined once each week and calculated as g food/mouse/week. Food efficiency [body weight gained (g)/food consumed (g) X 100] was not calculated by the study authors; however, food conversion ratios [food consumed (g)/body weight gained (g)] were calculated over the first 13 weeks of treatment. The compound intake (mg/kg/day) was calculated for each concentration from the group mean food intake and body weight data.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not required and were not performed.

5. Blood was collected from the orbital sinus of 1 male and 1 female from each cage during treatment weeks 26, 52, 78, and 104. The animals were under light ether anesthesia and were not fasted prior to blood collection. EDTA was used as an anticoagulant. The CHECKED (X) parameters were examined.

a. Hematology

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	X	Red and white blood cell and platelet
	(Thromboplastin time)		morphology
	(Clotting time)		Red cell distribution width (RCDW)
	(Prothrombin time)		

* Minimum required for oncogenicity studies unless effects are observed, based on Subdivision F Guidelines.

b. Clinical chemistry

Clinical chemistry tests were not conducted and are not required for oncogenicity studies based on Subdivision F guidelines.

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6. Urinalysis

Urinalysis tests were not conducted and are not required for oncogenicity studies based on Subdivision F guidelines.

7. Sacrifice and pathology

Necropsies were done on all animals that died or were killed at unscheduled times during the treatment period. At scheduled study termination, all surviving animals were sacrificed by CO_2 asphyxiation and necropsied. The CHECKED (X) tissues from all groups were collected for histopathological examination. The eyes were preserved in Davidson's fixative; all other tissues were fixed in 10% buffered formalin. Tissue samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Kidney sections were also stained with Oil Red O (ORO) or Periodic Acid-Schiff reagent. Frozen sections of the livers were also examined after staining with (ORO). The lungs, liver, and kidneys from all animals in all groups were examined microscopically, and other listed tissues were examined from the control and high-dose groups. Any tissue showing a treatment-related change at the high dose was examined in all dose groups. All listed tissues were examined in all groups from mice that died or were killed at unscheduled times during the study. The (XX) organs from all animals killed at the scheduled sacrifice were weighed.

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

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

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II. RESULTS

A. OBSERVATIONS

1. Toxicity

There were no treatment-related increases in the incidences of clinical observations in treated animals compared to the control groups.

2. Mortality

The mortality at selected times during the study and percent survival at study termination is given in Table 2. No treatment-related decreases in survival were seen in the 104-week study. The survival at study termination in high-dose males was 44% compared to 38% in the combined control groups. Survival in high dose females was 52% compared to 59% in the combined female control groups.

TABLE 2. Mortality and percent survival of male and female mice fed Fluazinam for 104 weeks

Study interval	Dietary concentration (ppm)				
	0 (n = 104)	1 (n = 52)	10 (n = 52)	100 (n = 52)	1000 (n = 52)
Males					
Weeks 1 - 52	10	4	4	11	2
Weeks 53 - termination	54	31	25	21	27
% Survival	38	33	44	38	44
Females					
Weeks 1 - 52	3	3	0	1	2
Weeks 53 - termination	40	18	19	18	23
% Survival	59	60	63	63	52

Data taken from p. 37, MRID 42208405

B. BODY WEIGHT

Body weight and body weight gain data are summarized in Table 3. There were no treatment-related decreases in body weight or body weight gain seen during the study. The body weights of the treated males were comparable to the control groups. The body weights of treated females were comparable or slightly higher than the control weights especially near the end of the study. The mean body weights of females over the last 3 weeks of the study was shown to be significantly higher than that of the combined female control groups by about 10% ($p<0.01$).

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TABLE 3. Group mean body weights and body weight gains in male and female mice fed Fluazinam for 104 weeks (g)						
Body weight or weight gain measured on test week	Dietary concentration (ppm)					
	0	0	1	10	100	1000
	Males					
Body wt. at week 0	30	30	29	30	30	30
Body wt. at week 52	45	44	44	45	45	45
Body wt. at week 104	43	43	42	42	44	43
Wt. gain weeks 0-104[a]	13	13	13	12	14	13
	Females					
Body wt. at week 0	23	23	23	23	23	23
Body wt. at week 52	34	35	37	36	36	36
Body wt. at week 104	35	37	38	38	39	39
Wt. gain weeks 0-104[a]	12	14	15	15	16	16

Data taken from Table 3, pp. 60-69, MRID 42208405.
[a]Calculated by reviewer.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

There were no significant differences in food consumption in any treated group compared to the control groups at any week during the study.

2. Compound consumption

The compound consumption was calculated by the study authors from the food consumption and body weight data. The results are given in Table 1.

3. Food efficiency

The food efficiency was not calculated by the study authors; however, food conversion ratios, which vary inversely to the food efficiency, were calculated for treatment weeks 1 through 13. The results were similar in all groups (males: 50.8, 52.2, 48.1, 46.4, 51.5; females: 57.8, 53.5, 49.8, 53.9, 59.7 at 0, 1, 10, 100, and 1000 ppm, respectively).

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D. BLOOD WORK

1. Hematology

There were no large dose-related changes seen in the hematology parameters. The platelet counts were elevated about 20% ($p<0.01$) in females at 1000 ppm compared to the controls after 78 and 104 weeks of treatment, but were not affected in males at these time points. With this one exception, changes seen in hematology parameters in treated animals compared to the combined control groups were small in magnitude (10% or less), were not generally consistent from one measurement period to the next, or did not show a clear dose effect. Some changes were sporadically significant statistically, but were well within normal values.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

The final body weights and selected organ weights are summarized in Table 4. The group mean liver weight of males was increased by about 45% ($p<0.01$) at 1000 ppm compared to the control group, and the mean liver weights of females were increased by 15% and 30% ($p<0.01$) at 100 and 1000 ppm. The liver weights were adjusted for the final bodyweight. There were no other significant changes in organ weights in treated animals compared to the controls.

TABLE 4. Group mean organ and final body weights in male and female mice fed Fluazinam for 104 weeks (grams)

Organ	Dietary concentration (ppm)				
	0 (combined)	1	10	100	1000
Males					
Final body	43.49	41.82	42.38	44.07	43.03
Liver[b]	2.607	2.923	3.056	2.523	3.789** (45%)[a]
Females					
Final body	35.77	36.74	36.51	37.21	36.65
Liver[b]	1.745	1.817	1.760	2.008** (15%)	2.264** (30%)

Data taken from Table 7, pp. 82-83, MRID 42208405
[a](Percent difference from the control)
[b]Liver weight was adjusted for the final bodyweight as covariate.
**$p<0.01$, Significantly different from the control.

2. Gross pathology

Selected macroscopic findings are summarized in Table 5. Findings with increased incidences in treated males compared to the control groups occurred in the liver at

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1000 ppm. The effects in males include an increased incidence of mass or masses (controls, 32%; 1000 ppm, 73%, $p<0.01$), an increased incidence of irregular or pitted surfaces (controls, 0%; 1000 ppm, 17%, $p<0.01$), an increased incidence of pale area or areas (controls, 6%; 1000 ppm, 23%, $p<0.05$), an increased incidence of pale liver (controls, 20%; 1000 ppm, 40%, $p<0.05$), and an increased incidence of lobular markings accentuated (controls, 2%; 1000 ppm, 19%, $p<0.01$). In high-dose males, an increased incidence of lung mass or masses (controls, 24%; 1000 ppm. 46%, $p<0.05$) was also observed. In females, the only toxicologically significant change in the liver was an increase in the incidence of irregular or pitted liver surfaces (controls, 5%; 1000 ppm, 17%, $p<0.05$). An increase in the incidence of pale areas of the liver in treated female mice was not dose-related and was considered to be of equivocal toxicological significance. An increase in the incidence of uterine masses was also found in high-dose females (controls, 13%; 1000 ppm, 29%, $p<0.05$). No other macroscopic findings had increased incidences in treated animals compared to the controls.

Table 5. Macroscopic findings in male and female mice fed Fluazinam for up to 104 weeks

Organ or tissue/lesion	Dietary concentration (ppm)					
	0	0	1	10	100	1000
Males (n = 52)						
Liver/mass(es)	12	21(32%)	20	20	18	38**(73%)
Liver/irregular or pitted surface	0	0(0%)	2	2	1	9**(17%)
Liver/pale area or areas	2	4(6%)	4	5	7	12*(23%)
Liver/pale	11	10(20%)	8	8	6	21*(40%)
Liver/lobular markings accentuated	2	0(2%)	0	0	0	10**(19%)
Lung/mass(es)	13	12(24%)	17	20	19	24*(46%)
Female (n = 52)						
Liver/mass(es)	5	2	5	6	6	3
Liver/irregular or pitted surface	3	2(5%)	3	0	3	9*(17%)
Liver/pale area or areas	0	3(3%)	6*(12%)	1	7*(14%)	6*(12%)
Liver/pale	14	18	10	14	15	13
Lung/mass(es)	16	17	14	16	19	17
Uterus/mass or masses	7	7(13%)	6	4	9	15*(29%)

Data taken from Table 8, pp. 84-93, MRID 42208405

[a](Percent of animals with lesion, control groups were combined.)

*$p<0.05$, **$p<0.01$, Significantly different from controls; Fisher's exact test by the reviewer.

3. Microscopic pathology

a. Non-neoplastic

Selected microscopic findings in the mice are summarized in Table 6. Most of the findings that increased in treated animals compared to the combined control groups were in the liver. Liver effects seen in high-dose males, but not females, included increased incidences of basophilic hepatocytes (control, 12%; 100 ppm, 13%, NS; 1000 ppm 38%, $p<0.01$) and eosinophilic vacuolated hepatocytes (control, 1%; 100 ppm, 8%, $p<0.05$; 1000 ppm, 13%, $p<0.01$). Increased incidences of brown pigmented macrophages were seen in treated animals of both sexes compared to the control groups (males: control, 13%; 100 ppm, 27%, $p<0.05$; 1000 ppm, 62%, $p<0.01$; females: control, 15%; 1 ppm, 40%, $p<0.01$; 10 ppm, 21%, NS; 100 ppm, 38%, $p<0.01$; 1000 ppm, 50%, $p<0.01$). Incidences of minimal granulomatous hepatitis were increased in high-dose males (control, 11%; 1000 ppm, 25%, $p<0.01$). When considered as separate groups, the percentages of animals with findings among the mice that survived to study termination were generally higher than in animals that died or were killed during the study.

Increases in the incidences of cystic follicles in the thyroids were seen in high dose males (control, 23%; 1000 ppm, 52%, $p<0.01$) and high-dose females (control, 16%; 1000 ppm, 33%, $p<0.05$). The incidences of cystic thyroid follicles in females were significantly ($p<0.01$) decreased at other fluazinam concentrations compared to the control groups. The incidence of thymus hyperplasia was increased in high-dose females compared to the control groups (control, 5%; 1000 ppm, 21%, $p<0.01$) and was found only in the surviving animals at 1000 ppm.

The brain and spinal cords of all mice in the study were re-examined at a later date and the results submitted as an addendum to this study (MRID 44807220). The incidences of vacuolation of white matter in the cerebrum and in the cerebellum/pons/medulla were significantly increased at 1000 ppm compared to the controls in males (controls, 68% and 74%; 1000 ppm, 90%, $p<0.05$ and 94%, $p<0.01$ in the cerebrum and cerebellum/pons/medulla., respectively). In females, the incidence of white matter vacuolation was not significantly increased in the cerebrum and only slightly increased in the cerebellum/pons/medulla. (control, 92%; 100 and 1000 ppm, 100%, $p<0.05$). However, the severity of the lesions, which were graded "trace, minimal or moderate" by the study authors, in the cerebrum was increased in females at 1000 ppm compared to the controls (incidence of lesions graded "minimal" or "moderate": control, 8%; 1000 ppm 55%, $p<0.01$). Most lesions found at the lower concentrations were graded "trace"; only 12 females in the entire study were found to have brain lesions that graded "moderate" and none were found in males. The white matter vacuolation in the cerebrum of high-dose males that graded "minimal" was 32% ($p<0.01$) versus 0% in the control groups, and in the cerebellum/pons/medulla, 45% ($p<0.05$) of lesions were so graded in high-dose males compared to 26% in the combined controls. The incidence of

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white matter vacuolation in the spinal cord increased slightly in high-dose males (controls, 72%; 1000 ppm, 87%, $p<0.05$) but not in high-dose females and did not increase in severity at the high dose in either sex compared to the control groups.

TABLE 6. Non-neoplastic histopathology findings in male and female mice fed Fluazinam for up to 104 weeks

Organ/tissue	Dietary concentration (ppm)					
	0	0	1	10	100	1000
Males (total n = 52)						
Liver/ basophilic hepatocytes	6	6(12%)[a]	5	7	7(13%)	20**(38%)
Liver/ brown pigmented macrophages	9	5(13%)	6	5	14*(27%)	32**(62%)
Liver/ eosinophilic vacuolated hepatocytes	0	1(1%)	1	0	4*(8%)	7**(13%)
Liver/ granulomatous hepatitis (minimal)	7	4(11%)	5	6	10	13**(25%)
Thyroid/ cystic follicles	12/51[b]	12(23%)	13	7/51	13	27**(52%)
Brain, cerebrum/ vacuolation of white matter	38 0%[c]	33(68%) 0%	43 0%	40 0%	34 3%	47+(90%) 32%**
Brain, cerebellum, pons, medulla/ vacuolation of white matter	39 36%[c]	38(74%) 16%	43 21%	41 32%	42 33%	49++(94%) 45%*
Spinal cord/ vacuolation of white matter	37 38%[c]	38(72%) 16%	39 26%	33 21%	40 35%	45+(87%) 24%
Females (total n = 52)						
Liver/basophilic hepatocytes	1	4	3	2	2	2
Liver/brown pigmented macrophages	12	4(15%)[a]	21**(40%)	11(21%)	20**(38%)	26**(50%)
Liver/eosinophilic vacuolated hepatocytes	0	0	0	1	0	1
Liver/granulomatous hepatitis (minimal)	6	2(11%)	4	5	5	11(21%)
Thyroid/cystic follicles	9/50[b]	7(16%)	4	0**	0**	17*(33%)
Thymus/hyperplasia (minimal)	3	2/50(5%)	2/23	3/16*	0/21	10/48**(21%)
Brain, cerebrum/ vacuolation of white matter	48 8%[c]	46 11%	48 19%	51 16%	50 10%	51 55%**
Brain, cerebellum, pons, medulla/vacuolation of white matter	50 72%[c]	46(92%) 52%	49 49%	51 55%	52+ 48%	52+ 63%
Spinal cord/vacuolation of white matter	50 66%[c]	44 30%	45 40%	49 37%	51* 27%	43 28%

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Data taken from Table 10, pp. 113-145, MRID 42208405. Data on brain were taken from pp. 9-10, and Table 1, pp. 15-18, MRID 44807220 (addendum study to MRID 42208405).
[a](Percent of animals with lesion indicated, control groups were combined.)
[b]Number of animals examined if less than 52.
[c]Percentage of affected mice with brain lesions that were graded minimal or moderate.
*p<0.05, **p<0.01, Significantly different from controls, 2X2 chi square test by reviewer. The control groups were combined in the statistical analyses.
[+]p<0.05, [++]p<0.01, Significantly different from the control 1 in males, control 2 in females. Fisher's exact test by the study author.

b. Neoplastic

A summary of neoplasms seen in this study is given in Table 7. The total incidences of hepatocellular adenoma were significantly (p<0.05) increased in males at 1000 ppm compared to the combined control groups (controls, 14%; 1000 ppm, 33%, p<0.05), and there was also a significant positive trend from the control groups through the 1000 ppm male group (p<0.05). The incidence of hepatocellular carcinoma in males was increased at 1000 ppm compared to the control groups, but the increase was not statistically significant (control 17%; 1000 ppm, 33%, p = 0.09). The hepatocellular carcinoma incidences in males did show a significant positive trend (p<0.05). There were no differences in the incidences of hepatocellular neoplasms in treated and control female mice. The incidences of lung adenocarcinoma in female mice showed a statistically significant (p = 0.047) positive trend; however, the incidence at 1000 ppm was not significantly different from the combined control groups (controls, 10%, 1000 ppm 15%, NS). Except for histiocytic sarcomas, the incidence of no single type of uterine tumor was increased in treated mice at any dose level. For histiocytic sarcoma, a slight increase was observed at 1000 ppm (control, 2%; 1000 ppm, 10%).

TABLE 7. Neoplastic findings in male and female mice fed Fluazinam for up to 104 weeks[a]

Organ and/or neoplasm	Dietary concentration (ppm)					
	0	0	1	10	100	1000
Males (n=52)						
Liver/ hepatocellular adenoma	6	9[+](14%)[b]	12	9	7	17*(33%)
Liver/ hepatocellular carcinoma	9	9[+](17%)	8	7	7	17(33%)
Lung/ adenoma	1	2	1	6	8	6
Lung/ adenocarcinoma	10	4	10	6	5	6
Females (n=52, except for uterus)						
Liver/ hepatocellular adenoma	0	1	2	0	1	0
Liver/ hepatocellular carcinoma	1	0	1	1	1	0
Lung/ adenoma	1	6	5	4	5	4
Lung/ adenocarcinoma	4	6[+](10%)	2	5	3	8(15%)
Uterus, number examined	52	52	44	42	46	52
Uterus/endometrial sarcoma	0	1	0	0	1	0
Uterus/leiomyosarcoma	2	0	0	1	1	0

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TABLE 7. Neoplastic findings in male and female mice fed Fluazinam for up to 104 weeks[a]						
Uterus/lymphosarcoma	2	4	1	0	3	4
Uterus/histiocytic sarcoma	0	2(2%)	1	0	3	5(10%)

Data taken from pp. 40-42 and Table 9, pp. 94-112, MRID 42208405.
[a] Mice with both an adenoma and a carcinoma (liver) or adenocarcinoma (lung) were listed twice–once under adenoma and once under carcinoma (liver) or adenocarcinoma (lung).
[b] Percent of animals with tumor, control groups were combined.
*p<0.05, Significantly different from the control.
+p<0.05, Significant trend over 0 to 1000 ppm dietary concentrations.

Historical control data for liver cell tumors in male mice and for lung tumors in female mice were provided in the study report for 9 comparable studies started in the same testing laboratory in 1981-1983 with study durations of 97-108 weeks. See Table 8.

TABLE 8. Historical control data for liver cell tumors in male mice and for lung tumors in female mice (Percent incidence)									
Organ / neoplasm	Study number								
	1	2	3	4	5	6	7	8	9
Males									
Liver/ hepatocellular adenoma	4	13	14	12	18	21	23	27	17
Liver/ hepatocellular carcinoma	23	24	16	27	28	17	12	19	38
Females									
Lung/ adenoma	8	2	5	2	2	4	21	10	4
Lung/ adenocarcinoma	2	13	14	10	15	8	12	12	15

Data taken from pp. 41-42, MRID 42208405.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSION

The investigators concluded that fluazinam treatment at 1000 ppm compared to the controls resulted in increased liver weights and macroscopically observed liver lesions in both sexes. Increased liver weights were also seen in females at 100 ppm. Increased numbers of foci/areas of basophilic or eosinophilic hepatocytes were seen in males at 1000 ppm compared to the controls. Treatment related increased incidences of granulomatous hepatitis and brown pigmented macrophage aggregation were seen in males at 100 and 1000 ppm and in females at 1000 ppm.

The dietary concentration of 10 ppm was considered to be the no-observable- effect-level (NOEL) for treatment of mice with fluazinam (B-1216) for 104 weeks.

The central nervous systems of the animals were re-examined and the results reported in an addendum to the main study (MRID 44807220). Treatment-related increases in the incidences and severity of vacuolation of white matter occurred in the brains of males and increased severity of white matter vacuolation was seen in the brains of females at 1000 ppm compared to the control groups. No clear effect of treatment on the incidence or severity of white matter vacuolation in the spinal cord was seen in either sex, and no treatment-related effects were seen in mice at 1, 10, or 100 ppm.

Treatment of males at 1000 ppm was associated with an increase in benign liver cell tumors. No effects on tumor incidence were seen in males at 1, 10, or 100 ppm or in females at any concentration level. A reviewer of this study (James C. Killeen, Ph.D.; Ricerca Inc., Painesville, Ohio; comments dated 2/10/92; pp. 5-13 in Vol. 1 of the study report) commented that the statistical significance of the benign tumor incidence at 1000 ppm in males depended on the statistical test being applied, and the incidence of benign liver tumor in 1000 ppm males was only slightly higher than that in historical control animals. The reviewer concluded that it was not possible to arrive at a definitive conclusion regarding the association of the liver tumors in high-dose males with the test material when all of the evidence is evaluated.

B. REVIEWER'S DISCUSSION

No treatment-related clinical signs were noted in mice fed the test material for up to 104 weeks. There were no significant treatment-related effects on survival, food consumption, body weights, or body weight gain. Although some hematology parameters in treated animals were sporadically significantly different from the control groups, the differences were not consistent from one time point to another, did not show clear dose effects, and the differences were slight (±10%) or within the range of normal values. Platelet counts in high-dose females were increased about 20% ($p<0.01$) compared to the control groups at weeks 78 and 104, but these values are still within the normal range and of doubtful toxicological significance.

Group mean liver weights, which were adjusted for the final body weights, were increased in males by about 45% ($p<0.01$) and females by 30% ($p<0.01$) at 1000 ppm compared to the control groups. Liver weights were also increased in females by 15% ($p<0.01$) at 100 ppm, compared to the control groups, but were not affected in males at that concentration. Macroscopic examination during necropsy of high-dose males revealed increased incidences of liver masses (controls, 32%; 1000 ppm, 73%, $p<0.01$), areas of the liver with irregular or pitted surfaces (controls, 0%; 1000 ppm, 17%, $p<0.01$), pale liver areas (controls, 6%; 1000 ppm, 23%, $p<0.05$), and pale livers (controls, 20%; 1000 ppm, 40%, $p<0.05$). The only macroscopic finding in the livers of females was an increased incidence of irregular or pitted surfaces at 1000 ppm compared to the controls (controls, 5%; 1000 ppm, 17%, $p<0.05$). In males microscopic examination revealed increased incidences of liver areas containing basophilic (controls, 12%; 1000 ppm, 38%, $p<0.01$) and/or eosinophilic hepatocytes (controls, 1%; 100 ppm, 8%, $p<0.05$; 1000 ppm, 19%, $p<0.01$). Increased incidences of

granulomatous hepatitis were seen in high-dose animals of both sexes compared to the controls (males: controls, 11%; 100 ppm, 19%, NS; 1000 ppm, 37%, $p<0.01$; females: controls, 11%; 1000 ppm, 21%, $p<0.01$). Increased incidences of aggregates of brown pigmented macrophages in the livers of treated mice were seen compared to the control groups in males (controls, 13%; 100 ppm, 27%, $p<0.05$; 1000 ppm, 62%, $p<0.01$) and in females (controls, 15%; 1 ppm, 40%, $p<0.01$; 10 ppm, 21%, NS; 100 ppm, 38%, $p<0.01$; 1000 ppm, 50%, $p<0.01$). Granulomatous hepatitis and brown pigmented macrophages were seen primarily in the older animals that survived until the end of the study. The granulomatous hepatitis was minimal in severity, and the incidences of pigmented macrophages did not show a clear dose effect in females. No hyperplasia of the liver was seen in either sex at any dose. Although the findings seen during the microscopic examination confirmed the liver as a target of fluazinam toxicity and supported some of the macroscopic observations, they do not correlate with the increases in liver weight in either sex, and whether they constitute adaptive or adverse effects is questionable especially at the lower concentrations. The study authors considered the increased incidence of granulomatous hepatitis and other liver effects in treated animals to be an exacerbation of a spontaneous change seen in aging mice. The increases in the percentages of affected mice surviving until the end of the study compared to the percentages of affected mice that died earlier in the study support this conclusion.

Macroscopic examination showed an increased incidence of uterine masses in high-dose females compared to the controls (controls, 13%; 1000 ppm, 29%, $p<0.05$). This finding was not confirmed or supported by the histological examinations. The histological examination did show an increased incidence of hyperplasia in the thymus of high-dose females that only occurred in the high-dose mice surviving till week 104 (total animals: controls, 5%; 1000 ppm, 21%, $p<0.01$). No similar increase in thymic hyperplasia was seen in males. Increased incidences of cystic thyroid follicles were seen in high-dose males (controls, 23%; 1000 ppm, 52%, $p<0.01$) and high-dose females (controls, 16%; 1000 ppm, 33%, $p<0.01$). The toxicological significance of these findings is not known.

Increased incidences and severity of white matter vacuolation in the brains of high-dose mice were reported in an addendum study (MRID 44807220). The increased incidences of vacuolation of white matter in the cerebellum/pons/medulla were slight but statistically significant in high-dose males (control, 74%; 1000 ppm, 94%, $p<0.01$) and high-dose females (controls, 92%; 1000 ppm, 100%, $p<0.05$). Nearly all the animals that survived till the end of the study in all dose groups were found to have central nervous system white matter vacuolation graded "trace"; however, the severity of the cerebrum white matter vacuolation increased markedly at 1000 ppm. The incidence of lesions in the cerebrum graded "minimal" or "moderate" in the male control groups was 0%, but at 1000 ppm it was 32%, and in females about 10% of lesions in the cerebrum of control mice and 55% in high-dose females were so graded. The highest grade of brain lesions in males was "minimal" and 9/12 lesions in females graded "moderate" were seen at 1000 ppm.

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The lowest-observed-adverse-effect-level (LOAEL) seen in this study was 100 ppm (10.72 mg/kg/day for males, 11.72 mg/kg/day for females) based on increased incidences of brown pigmented macrophages in the liver of both sexes, increased incidences of eosinophilic vacuolated hepatocytes in males, and increased liver weights in females. A no-observed-adverse-effect-level (NOAEL) of 10 ppm (1.12 mg/kg/day for males, 1.16 mg/kg/day for females) was determined.

Treatment of CD®-1 mice for up to 104 weeks resulted in a significant increase in the hepatocellular adenoma incidence in high-dose males under the conditions of this study (controls, 14%; 1000 ppm 33%, $p<0.05$). The incidence of hepatocellular carcinoma was also increased in high-dose males, but the increase was not statistically significant by the tests applied by the study authors (controls, 17%; 1000 ppm, 33%, NS). No increases in hepatocellular tumor incidences were seen in treated females compared to the controls. Historical control data supplied with the study showed the hepatocellular adenoma incidence in males ranged from about 4% to 27% in mouse studies of similar duration, and the hepatocellular carcinoma incidence ranged from 12% to 38%.

This oncogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an oncogenicity study [OPPTS 870.4200 (83-2b)] in mice. An additional study has been done following this study with higher concentrations of fluazinam (see MRID 44807222).

C. STUDY DEFICIENCIES

There were no serious deficiencies in the study.

APPENDIX 1

Four-week Toxicity Study in Mice

Dose Selection Study in Mice

MRID NO.: 44807212

Study Type: Supplementary study for [870.4200 (§83-2b)]

Test Material: Technical fluazinam (96.3%, lot no. 8203)

Document No: 82/ISK036/067 and addendum: 82/ISK036/215

Submitted by: ISK Biosciences Corporation, 5970 Heisley Road, Suite 200 Mentor, Ohio 44060.

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

Testing Facility: Life Science Research, Stock, Essex, CM4 9PE, England

Citation: Amyes, S.J., et. al. (1983) B-1216: Four-week toxicity study in mice. Contains: final report; dietary assays - addendum 1; and addendum 2.

Report Date: June 9, 1983

Methods/ Results/Conclusion:

Test Animals: CD-1 mice, 39-46 days old at study initiation

Group Size: 10 males, 10 females

Test Concentrations: 0, 10, 50, 250, or 3000 ppm in the diet

Test substance intake: Males: 0, 1.5, 7.6, 36, and 438; females: 0, 1.6, 8.2, 43, and 472 mg/kg/day

Duration: 4 weeks

Results:

Clinical signs: No treatment-related clinical signs were seen.

Mortality: No treatment-related deaths occurred.

Body weight: Body weight gains in treated animals were slightly less than that of the control groups (males: control, 11 g, 10 ppm, 9 g; 50 ppm, 9 g, $p<0.05$; 250 ppm, 8 g; 3000 ppm, 8 g, $p<0.01$; females: control, 6 g; 10 ppm, 4 g, $p<0.01$; 50 ppm, 5 g, NS; 250 ppm, 4 g, $p<0.01$; 3000 ppm, 5 g, $p<0.05$). The change in body weight gain in treated animals compared to the control group was slight, occurred sporadically during the study, and did not show a clear dose dependency especially in females.

Food consumption: Group mean food consumption in treated animals was comparable to the control group

Clinical pathology: The platelet counts were increased in males by about 17% ($p<0.01$) at 3000 ppm compared to the control. Other hematology parameters measured during the 3rd week of treatment including hematocrit, hemoglobin, red cell, total white cell, and differential white cell counts, mean corpuscular hemoglobin concentration, and mean corpuscular volume were not changed in a dose-related manner. The total blood cholesterol was increased by 23% in males and by 30% in females at 3000 ppm compared to the controls, and the blood glucose and phospholipids were increased by 32 and 30%, respectively, in high-dose females. All the hematology and clinical chemistry changes are within the normal ranges for CD-1 mice making the toxicological significance of the changes questionable.

Organ weights: The group mean absolute liver weights were increased by 22% in males and by 26% in females at 3000 ppm compared to the controls ($p<0.01$). The mean liver weights relative to body weight were also increased by 20% and 25% in high-dose males and females, respectively ($p<0.01$). Absolute kidney weights were increased by 14%, and the relative kidney weights were increased by 12% and 15% at 250 and 3000 ppm, respectively, in females, but not in males ($p<0.01$).

Histopathology: Increased incidences of periacinar hepatocytic hypertrophy were seen in high-dose males compared to the control group (control, 1/10; 250 ppm, 6/10, NS; 3000 ppm, 10/10, $p<0.01$). Increases in the incidences of periacinar fine fatty vacuolation in high-dose mice were not statistically significant, but may be toxicologically relative (males: control, 1/10; 3000 ppm, 6/10, NS; females: control, 0/10; 3000 ppm, 4/10, NS). In an addendum to the study, the histopathology of the brain of treated animals compared to the controls was re-examined for vacuolation of white matter. No treatment-related changes were found.

Conclusions: The study authors concluded there were no treatment-related findings at 50 ppm and minimal toxicity at 250 ppm to fluazinam (B-1216).

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Core classification: This study is classified as Acceptable/non-guideline. It was originally performed for guideline 82-1a for which the study is not acceptable since it was not a 90-day study; however, its use as a dose range-finding study is appropriate.

RAB2300:fluazi14.030

Attachment #2

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

Carcinogenicity Study in CD®-1 Mice (1988)

Reference:

Mayfield, R., S. Burton, D. Crook et al. (1988) Fluazinam technical (B-1216): potential carcinogenicity study in dietary administration to mice for 104 weeks. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 9/87264, September 29, 1988. MRID 42208405. Unpublished.

Mayfield, R. (1996) Amendment and addendum to report no ISK 9/87264: technical fluazinam potential carcinogenicity to mice (MRID 42208405). Huntingdon Life Sciences Ltd., P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 9/87264, December 19, 1996. MRID 44807220. Unpublished.

Amyes, S.J., S.M. Macrae, J.C. Whitney (1983) B-1216: Four-week toxicity study in mice. Life Science Research, Stock, Essex, CM4 9PE, England, Document Nos. 82/ISK036/067 and 82/ISK036/215, June 9, 1983. MRID 44807212. Unpublished.

A. Experimental Design

In a carcinogenicity study (MRID 42208405), Fluazinam (95.3% a.i., lot no. 8412-20) was administered to groups of 52 male and 52 female CD®-1 mice in the diet at concentrations of 0, 0, 1, 10, 100 or 1000 ppm. There were 2 control groups. The test diets were given for 104 weeks. These concentrations resulted in mean daily compound intakes of 0.12, 1.12, 10.72 and 107 mg/kg/day for 1 ppm, 10 ppm, 100 ppm, and 1000 ppm, respectively, for males and 0.11, 1.16, 11.72 and 117 mg/kg/day, respectively, for females. Additional microscopic review of brain and spinal cord was presented in MRID 44807220. A four-week-range finding study (MRID 44807212) using 0, 10, 50, 250 or 3000 ppm in the diet was also conducted.

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

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of fluazinam in the male or female mice [(4)].

[(4)] Brunsman, L.L. (2000) Fluazinam qualitative risk assessment based on Sprague-Dawley rat and CD-1 mouse dietary studies. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), December 13, 2000. Tox Doc. No. 014401. pp. 7-8.

A summary of the hepatocellular neoplasms seen in the male mice in this study is given in Table 4. The total incidences of hepatocellular adenoma were significantly ($p<0.05$) increased in males at 1000 ppm compared to the combined control groups (combined controls, 16%; 1000 ppm, 34%), and there was also a significant positive trend from the control groups through the 1000 ppm male group ($p<0.05$). The total incidences of hepatocellular carcinoma were significantly ($p<0.05$) increased in males at 1000 ppm compared to the combined control groups (combined controls, 19%; 1000 ppm, 34%), and there was also a significant positive trend from the control groups through the 1000 ppm male group ($p<0.01$). The total incidences of combined hepatocellular adenomas and/or carcinomas were significantly ($p<0.01$) increased in males at 1000 ppm compared to the combined control groups (combined controls, 33%; 1000 ppm, 62%), and there was also a significant positive trend from the control groups through the 1000 ppm male group ($p<0.01$). There were no compound-related hepatocellular tumors in the treated female mice in this study.

The incidence of lung adenoma in female mice at 1000 ppm was not significantly different from the combined control groups (combined controls, 7%, 1000 ppm, 8%). The incidences of lung adenocarcinoma in female mice,however, showed a statistically significant ($p = 0.047$) positive trend, but the incidence at 1000 ppm was not significantly different pair-wise from the combined control groups (combined controls, 10%, 1000 ppm 15%). For lung adenomas in female mice, the range of the percent incidence in the historical control data was 2 to 21% and the mean was 6.4% [5]. For lung adenomas in female mice, the percent incidence in the female 1000 ppm group in this study (8%) did not exceed the highest percent incidence in the historical control data (21%) and was similar to the mean percent incidence in the historical control data (6.4%). For lung adenocarcinomas in female mice, the range of the percent incidence in the historical control data was 2 to 15 % and the mean was 11.2% [5]. The percent incidence in the female 1000 ppm group in this study (15%) did not exceed the highest percent incidence in the historical control data (15%) and was similar to the mean percent incidence in the historical control data (11.2%). Except for histiocytic sarcomas, the incidence of no single type of uterine tumor was increased in treated mice at any dose level when compared to at least one of the control groups. For histiocytic sarcoma, a slight increase was observed at 1000 ppm (combined controls, 2%; 1000 ppm, 10%). The CARC did not consider either the lung adenocarcinomas or the uterine histiocytic sarcomas observed in the treated female mice in this study to be treatment-related..

[5] Nine studies performed at Huntingdon Research Centre, Huntingdon, England; started between 1981 and 1983; studies of 97-108 weeks duration; MRID 42208405, p. 42.

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Table 4. Fluazinam - 1988 CD-1 Mouse Study - Males

Male Hepatocellular Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)

	Dose (ppm)				
	0	1	10	100	1000
Adenomas (%)	15/94 (16)	12/48 (25)	9/48 (19)	7/41 (17)	17[a]/50 (34)
p =	0.012*	0.142	0.421	0.527	0.013*
Carcinomas (%)	18/94 (19)	8/48 (17)	7/48 (15)	7/41 (17)	17[b]/50 (34)
p=	0.006**	0.454	0.334	0.490	0.039*
Combined (%)	31[c]/94 (33)	18[c]/48 (38)	15[d]/48 (31)	12[c]/41 (29)	31[e]/50 (62)
p =	0.000**	0.361	0.496	0.415	0.001**

from: Brunsman, L.L. (2000) Fluazinam qualitative risk assessment based on Sprague-Dawley rat and CD-1 mouse dietary studies. Memorandum from Lori L. Brunsman (HED) to Edwin Budd (HED), December 13, 2000. Tox Doc. No. 014401. p. 9.

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

[a]First adenoma observed at week 56, dose 1000 ppm.
[b]First carcinoma observed at week 56, dose 1000 ppm.
[c]Two animals in each of the control, 1 and 100 ppm dose groups had both an adenoma and a carcinoma.
[d]One animal in the 10 ppm dose group had both an adenoma and a carcinoma.
[e]Three animals in the 1000 ppm dose group had both an adenoma and a carcinoma.

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

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Historical control data for hepatocellular adenomas and carcinomas in male CD-1 mice were provided by the applicant in several separate submissions. The data have been combined and summarized below (Table 5).

TABLE 5. Historical control data for hepatocellular adenomas and carcinomas in male CD-1 mice [+] **(Percent incidence)**

Laboratory:	1981-1983[1][5] Huntingdon Research Centre (England)	1986-1988[2] Huntingdon Research Centre (England)	1991-1993[3] Huntingdon Research Centre (England)	1987-1993[4] Eye Research Centre (England)	1994-1996[4] Eye Research Centre (England)
No. of Studies:	9	9	12	12	12
Duration:	97 - 108 weeks	92 - 104 weeks	80 - 96 weeks	95 - 104 weeks	102 - 106 weeks
Adenomas					
Range	4 - 27%	8 - 23%	8 - 34%	0 - 31%	9 - 40%
Mean	16.6%	12.0%	15.9%	11.8%	21.0%
Carcinomas					
Range	12 - 38%	5 - 13%	2 - 16%	4 - 17%	2 - 15%
Mean	22.7%	8.0%	8.6%	9.5%	8.0%
Combined Aden/Carcin					
Range	------	------	------	4 - 42%	15 - 42%
Mean	------	------	------	20.8%	27.6%

[+] Uncensored Data.
[1] MRID 42208405, p. 41
[2] MRID 44807222, p. 2207
[3] MRID 44807222, p. 37
[4] MRID 45201301, pp.27-28
[5] Start date of study

Since the study under discussion (MRID 42208405) was initiated in 1985 at Huntingdon Research Centre (England), the most directly applicable historical control data is that from the same laboratory for the years 1981-1983 and 1986-1988 (first 2 columns in Table 5). For hepatocellular adenomas in the livers of male mice, the range of the percent incidence in the 1981-1983 historical control data (uncensored data) was 4 to 27% and the mean was 16.6% and in the 1986-1988 historical control data (uncensored data) was 8 to 23% and the mean was 12.0% . The percent incidence in the male 1000 ppm group (censored data) in this study (34%) exceeded the highest percent incidence in the historical control data for 1981-1983 (27%) and for 1986-1988 (23%). For hepatocellular carcinomas in the livers of male mice, the range of the percent incidence in the 1981-1983 historical control data (uncensored data) was 12 to 38% and the mean was 22.7% and in the 1986-1988 historical control data (uncensored data) was 5 to 13% and the mean was 8.0%. The percent incidence in the male 1000 ppm group (censored data) in this study (34%) did not exceed the highest percent incidence in the historical control data for 1981-1983 (38%), but did exceed the highest percent incidence in the historical control data for 1986-1988 (13%).

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C. Non-neoplastic Lesions

Statistically significant ($p<0.01$) increased incidences of several histopathological lesions in the liver of 1000 ppm male mice were observed in this study. These lesions included basophilic hepatocytes (combined controls, 12%, 1000 ppm, 38%), brown pigmented macrophages (combined controls, 13%, 1000 ppm, 62%), eosinophilic vacuolated hepatocytes (combined controls, 1%, 1000 ppm, 13%) and (minimal) granulomatous hepatitis (combined controls, 11%, 1000 ppm, 25%). The CARC noted that if the lesion described as "basophilic hepatocytes" were equivalent to "basophilic foci", this lesion might possibly be considered to be a pre-neoplastic effect of the test material. Brown pigmented macrophages (27%) and eosinophilic vacuolated hepatocytes (8%) were also statistically significantly ($p<0.05$) increased in 100 ppm male mice. In female mice, a statistically significant ($p<0.01$) increased incidence of brown pigmented macrophages was observed in the liver at 100 ppm and 1000 ppm (combined controls, 15%, 100 ppm, 38%, 1000 ppm, 50%).

A statistically significant increased incidence of cystic follicles of the thyroid gland was observed in male mice at 1000 ppm (combined controls, 23%, 1000 ppm, 52%, $p<0.01$) and in female mice at 1000 ppm (combined controls, 16%, 1000 ppm, 33%, $p<0.05$). The CARC determined that these lesions in the thyroid gland of the male and female mice in this study were unrelated to and did not support the finding of thyroid gland tumors in the rat study on fluazinam (MRID 42248620).

Treatment-related increases in the incidence and severity of vacuolation of the white matter of the brain of male mice and in the severity of vacuolation of the white matter of the brain of female mice were observed at 1000 ppm in this study.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CARC considered the dosing in this study to be adequate but not excessive for assessment of carcinogenic potential. This determination was based on the liver and brain toxicity observed in the male and female mice in this study at 1000 ppm. The group mean liver weights (adjusted for body weight) were statistically significantly ($p<0.01$) increased in males and females by 45% and 30%, respectively, at 1000 ppm and also in females by 15% at 100 ppm ($p<0.01$). Microscopic examination of livers also demonstrated several treatment-related lesions in males and females that were described above under "Non-neoplastic Lesions". Treatment-related increases in the incidences and severity of vacuolation of white matter of the brain was observed in males at 1000 ppm and increased severity of white matter vacuolation was seen in the brains of females at 1000 ppm compared to the control groups. No clear effect of treatment on the incidence or severity of white matter vacuolation in the spinal cord was seen in either sex. No treatment-related effects on the nervous system were seen at 1, 10 or 100 ppm.

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