US EPA ARCHIVE DOCUMENT

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

Edwin Budd, Date 2/16/01

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): Fluazinam Technical (purity, 97.0% a.i.)

SYNONYMS: B1216; 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine; IKF-1216; PP192

CITATION: Chambers, P.R. (1996) Technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (vols. 1-13). Contains: final report and addendums 1 & 2. Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671, December 19, 1996. MRID 44807222. Unpublished.

Chambers, P.R. (1998) Addendum report to: Technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (vols. 1-13). Contains: final report and addendums 1 & 2. Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671, August 28, 1998. MRID 44807221. Unpublished.

Gopinath, C. (2000) Pathology Working Group (PWG) report on liver tumours in study no. ISK 50/950671 - Technical fluazinam: potential tumorigenic effects in prolonged dietary adeministration to mice (MRID#s 4480722, and 44007221). Huntingdon Life Sciences LTD, Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671 PWG, August 24, 2000. MRID 45201301. Unpublished.

Chambers, P.R., T. Gardner, D. Crook, et. al. (1994) Technical fluazinam: toxicity to mice by dietary administration for 4 weeks, contains: final report and addendums 1 through 5. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 49/921049, March 7, 1994. MRID 44807211. Unpublished.

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

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

EXECUTIVE SUMMARY: In a carcinogenicity study (MRID 44807222, 44807221), technical grade Fluazinam (97.0% a.i., lot no. 1030/91) was administered to groups of 50 male and 50 female Crl:CD®-1 mice in the diet at concentrations of 0, 1000, 3000, or 7000 ppm. The test diets were given for 97 weeks to females and for 104 weeks to males. These concentrations resulted in mean daily compound intakes of 126, 377, and 964 mg/kg/day for males and 162, 453, and 1185 mg/kg/day for females for 1000 ppm, 3000 ppm, and 7000 ppm, respectively. Twenty mice were added to the control and 7000 ppm groups for a satellite study in which the mice were killed and necropsied after treatment for 78 weeks. A Pathology Working Group (PWG) report presenting revised incidences for hepatocellular tumors in the male mice in this study was later submitted (MRID 45201301). A four-week range-finding study in mice was also conducted using 0, 3000, 5000, and 7000 ppm in the diet (MRID 44807211).

Treatment with fluazinam resulted in a significant decrease in survival in females at 7000 ppm (control, 58%; 7000 ppm, 26%, $p < 0.01$). All females were terminated after 97 weeks of treatment because of low survival at the high-dose. At 7000 ppm, body weight gain was decreased in males during weeks 4-36 by 32% ($p < 0.01$) and food conversion ratios over weeks 9-13 were increased by 86% compared to the controls indicating decreased efficiency of food utilization. At termination, reltive liver/body weight ratios were increased in males by 54%, 113% and 182% and in females by 21%, 45%, and 109% at 1000, 3000, and 7000 ppm, respectively, compared to the controls ($p<0.01$). Microscopic examination showed increased incidences of altered hepatocyte foci at all concentration levels in males and in high-dose females (males: control, 12/50; 1000 ppm 24/50, $p<0.05$; 3000 ppm, 36/50; 7000 ppm, 33/50, $p<0.01$; females: control, 3/50; 7000 ppm 15/50, $p<0.01$). Incidences of hepatocyte enlargement, pale or vacuolated hepatocyte cytoplasm, and brown pigmented macrophage aggregates were increased in all treated males and females compared to the control groups ($p<0.01$). The pigmented macrophage aggregates also increased in severity from 0-22% of lesions in the controls to 41-58% of lesions at 7000 ppm graded "moderate" or "marked." Incidences of brown pigmented centrilobular hepatocytes and parenchymal inflammatory cells increased in males at 3000 ppm (both 6/50, $p<0.05$) and 7000 ppm (11-16/50, $p<0.01$) compared to the controls (0-1/50). Males were more sensitive to the hepatotoxic effects of fluazinam than females. Incidences of vacuolation of white matter in the brains of all treated animals were increased compared to the controls ($p<0.01$). Vacuolation of white matter was also increased in the cervical spinal cord of males at 3000 and 7000 ppm (control, 18/50; 3000 ppm, 37/50, $p<0.05$; 7000 ppm, 46/50, $p<0.01$) and marginally in females (control, 37/50; 3000 and 7000 ppm, 45/50, NS). The severity of the vacuolation of white matter in the brain and spinal cord increased with increasing dose from 0% graded "moderate" or "marked" in the controls to 33-60% of lesions at 7000 ppm. Incidences of left atrial thrombus in the hearts of high-dose males and females were increased compared to the controls and contributed to the unscheduled deaths of about 46% of high-dose males and 30% of high-dose females during the study.

The LOAEL is 1000 ppm in the diet (126 mg/kg/day for males; 162 mg/kg/day for females), based on increased liver weights in males and females and on histopathologic changes in the liver and brain in males and females. A NOAEL was not determined (<1000ppm).

In this study, there were no statistically significant positive trends for hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the male mice. For the mid-dose male mice (3000 ppm), however, there was a statistically significant increase by pair-wise comparison with the controls for hepatocellular adenomas (47% vs 17% in controls) and for combined hepatocellular adenomas/carcinomas (49% vs 18% in controls). For the high-dose male mice (7000 ppm), there was no statistically significant increase by pair-wise comparison with controls for hepatocellular adenomas or carcinomas when considered separately, but there was a statistically significant increase for combined adenomas/carcinomas (33% vs 18% in controls). The incidence of hepatocellular adenomas at the mid-dose level for males (47%) exceeded the highest incidence in the historical control data for 1991-1993 (8-34%) and for 1987-1993 (0-31%), but the incidence at the high-dose level for males (30%) did not exceed the highest incidences in the comparable historical control data. Similarly, the incidence of combined hepatocellular adenomas/carcinomas at the mid-dose level for males (49%) exceeded the highest incidence in the historical control data for 1987-1993 (4-42%), but the incidence at the high-dose level for males (33%) did not exceed the highest incidences in the comparable historical control data. For the male mice in this study, the tumorigenic response did not appear to be dose-related because the response at 7000 ppm was less than that observed at 3000 ppm. The highest dose level tested for the male mice in this study was considered to be adequate but not excessive. In this study, hepatocellular tumors were also observed in the female mice at the high-dose (7000 ppm). These tumors, however, occurred at an excessively toxic dose which may have resulted in indirect effects that may not have been present at lower doses. There was a treatment-related increased mortality for the high-dose females in this study. Although a statistically significant positive trend was observed for combined hepatocellular adenomas/carcinomas for the female mice in this study, this calculation included the response at 7000 ppm. At the next lower dose level (3000 ppm), there was no statistically significant pair-wise increase in hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the female mice in this study.

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.

NOTE--In the four-week range-finding study in mice (MRID 44807211), treatment-related increased incidences and severity of vacuolation of white matter of the brain were seen at four weeks in males at 3000, 5000, and 7000 ppm and in females at 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the spinal cord were also seen in males at 5000 and 7000 ppm. The LOAEL for vacuolation of white matter in the brain of male mice in this four-week study was 3000 ppm (555 mg/kg/day; lowest dose level tested). A NOAEL was not demonstrated for this effect (i.e. NOAEL <555 mg/kg/day).

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

RAB3001:44807222.der

Attachment #1

Original DER prepared for this study by Oak Ridge National Laboratory

DATA EVALUATION REPORT

FLUAZINAM (B1216)

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

MRID 44807222, 44807221, and 448072 11

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Chemical Hazard Evaluation Group
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Oak Ridge, TN 37830
Task Order No. 99-51L

Primary Reviewer: A. A. Francis, M.S., D.A.B.T.	Signature: Date:	Robert N. Ross for A.A. Francis DEC 2 9 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 4/6/00
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): Fluazinam Technical (purity, 97.0% a.i.)

SYNONYMS: B1216; 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine; IKF-1216; PP192

CITATION: Chambers, P.R. (1996) Technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (vols. 1-13). Contains: final report and addendums 1 & 2. Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671, December 19, 1996. MRID 44807222. Unpublished.

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Chambers, P.R., T. Gardner, D. Crook, et. al. (1994) Technical fluazinam: toxicity to mice by dietary administration for 4 weeks, contains: final report and addendums 1 through 5. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 49/921049, March 7, 1994. MRID 44807211. 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 44807222, 44807221, 44807211), technical grade Fluazinam (97.0% a.i.) was administered to groups of 50 male and 50 female

Crl:CD®-1 mice in the diet at concentrations of 0, 1000, 3000, or 7000 ppm. The test diets were given for 97 weeks to females and for 104 weeks to males. These concentrations resulted in mean daily compound intakes of 126, 377, and 964 mg/kg/day for males and 162, 453, and 1185 mg/kg/day for females for 1000 ppm, 3000 ppm, and 7000 ppm, respectively. Twenty mice were added to the control and 7000 ppm groups for a satellite study in which the mice were killed and necropsied after treatment for 78 weeks. A four-week range-finding study in mice was also conducted using 0, 3000, 5000, and 7000 ppm in the diet (MRID 44807211).

Treatment with Fluazinam resulted in a significant decrease in survival in females at 7000 ppm (control, 58%; 7000 ppm, 26%, $p < 0.01$). All females were terminated after 97 weeks of treatment because of low survival at the high-dose. At 7000 ppm, body weight gain was decreased in males during weeks 4-36 by 32% ($p < 0.01$) and food conversion ratios over weeks 9-13 were increased by 86% compared to the controls indicating decreased efficiency of food utilization. At termination, reltive liver/body weight ratios were increased in males by 54%, 113% and 182% and in females by 21%, 45%, and 109% at 1000, 3000, and 7000 ppm, respectively, compared to the controls ($p<0.01$). Microscopic examination showed increased incidences of altered hepatocyte foci at all concentration levels in males and in high-dose females (males: control, 12/50; 1000 ppm 24/50, $p<0.05$; 3000 ppm, 36/50; 7000 ppm, 33/50, $p<0.01$; females: control, 3/50; 7000 ppm 15/50, $p<0.01$). Incidences of hepatocyte enlargement, pale or vacuolated hepatocyte cytoplasm, and brown pigmented macrophage aggregates were increased in all treated males and females compared to the control groups ($p<0.01$). The pigmented macrophage aggregates also increased in severity from 0-22% of lesions in the controls to 41-58% of lesions at 7000 ppm graded "moderate" or "marked." Incidences of brown pigmented centrilobular hepatocytes and parenchymal inflammatory cells increased in males at 3000 ppm (both 6/50, $p<0.05$) and 7000 ppm (11-16/50, $p<0.01$) compared to the controls (0-1/50). Males were more sensitive to the hepatotoxic effects of fluazinam than females. Incidences of vacuolation of white matter in the brains of all treated animals were increased compared to the controls ($p<0.01$). Vacuolation of white matter was also increased in the cervical spinal cord of males at 3000 and 7000 ppm (control, 18/50; 3000 ppm, 37/50, $p<0.05$; 7000 ppm, 46/50, $p<0.01$) and marginally in females (control, 37/50; 3000 and 7000 ppm, 45/50, NS). The severity of the vacuolation of white matter in the brain and spinal cord increased with increasing dose from 0% graded "moderate" or "marked" in the controls to 33-60% of lesions at 7000 ppm. Incidences of left atrial thrombus in the hearts of high-dose males and females were increased compared to the controls and contributed to the unscheduled deaths of about 46% of high-dose males and 30% of high-dose females during the study.

The LOAEL is 1000 ppm in the diet (126 mg/kg/day for males; 162 mg/kg/day for females), based on increased liver weights in males and females and on histopathologic changes in the liver and brain in males and females. A NOAEL was not determined (<1000ppm).

Treatment of Crl:CD®-1 mice for up to 104 weeks resulted in treatment-related increases in hepatocellular adenoma in males at 3000 ppm and 7000 ppm (controls, 6/50; 3000 ppm, 22/50; 7000 ppm 16/50, $p<0.01$). The hepatocellular carcinoma incidence was also increased in high-dose males, but was not statistically significant (control, 1/50; 7000 ppm, 4/50, NS), and was within the range of historic control incidences. The incidences of combined hepatocellular

adenoma and/or carcinoma were also increased in males at 3000 ppm and 7000 ppm (control, 7/50; 3000 ppm, 23/50; 7000 ppm, 18/50, $p<0.01$). **The increased incidence of hepatocellular adenomas and of combined hepatocellular adenomas and/or carcinomas observed in male mice at 3000 ppm and 7000 ppm are considered to be treatment-related.** No significant increases in liver tumors were seen in treated females compared to controls.

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.

NOTE--In the four-week range-finding study in mice (MRID 44807211), treatment-related increased incidences and severity of vacuolation of white matter of the brain were seen at four weeks in males at 3000, 5000, and 7000 ppm and in females at 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the spinal cord were also seen in males at 5000 and 7000 ppm. The LOAEL for vacuolation of white matter in the brain of male mice in this four-week study was 3000 ppm (555 mg/kg/day; lowest dose level tested). A NOAEL was not demonstrated for this effect (i.e. NOAEL <555 mg/kg/day).

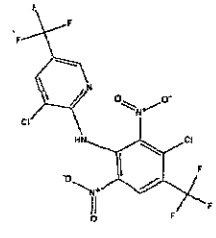
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Fluazinam Technical

   Description: yellow powder
   Lot/Batch #: 1030/91
   Purity: 97.0% a.i.
   Stability of compound: stable for the duration of the study.
   CAS #: 79622-59-6
   Received from: Ricerca Inc. (Sponsor's Monitoring Laboratory)
   Storage: 4^0 C in the dark
   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: Crl:CD®-1 (ICR) BR
Age and weight at study initiation: age: approximately 7 weeks; weight: males, 24 - 35 g; females, 19 - 29 g
Source: Charles River Breeding Laboratories, Portage, MI
Housing: Two mice of the same sex were housed in solid bottom polyproplyene cages with stainless steel wire tops for the duration of the study. Autoclaved sifted sawdust was used as bedding.
Diet: powdered SDS Rat and Mouse No. 1 modified maintenance diet, *ad libitum*
Water: tap water, *ad libitum*
Environmental conditions:
Temperature: 21 ± 2°C
Relative humidity: 55 ± 10%
Ventilation: not supplied
Light cycle: 12 hours light: 12 hours dark
Acclimation period: 21 days

B. STUDY DESIGN

1. In life dates - Start: October 1, 1992; end: October 3, 1994

2. Animal assignment

Animals were 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
1 - Control	0	0	0	70[b]	68[b]
2	1000	126	162	50	50
3	3000	377	453	50	50
4	7000	964	1185	70[b]	68[b]

Data taken from pp. 18, 19, 31, and Table 5, pp. 67-70, MRID 44807222.
[a]Daily dietary Fluazinam consumption was calculated from the group mean food consumption and body weight data.
[b]Groups of 20 males and 20 females were reserved from test groups 1 (control) and 4 (7000 ppm)for a satellite study in which the mice were killed after 78 weeks of treatment and subjected to rapid histopathological examination of the liver followed by subsequent histopathological examination of additional (required) tissues. The female groups were reduced by two animals each because of a technical error early in the study.

Main study male mice were treated for 104 weeks. Main study female mice were terminated after 97 weeks of treatment due to 75% mortality in the high-dose (7000 ppm) group.

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3. Dose selection:

The concentrations used in this study were based on 2 previous studies, a 104-week oncogenicity study and a 4-week range-finding study. In the 104-week study, groups of CD®-1 mice were given dietary concentrations of 0, 1, 10, 100, or 1000 ppm fluazinam (MRID 42208405). Increased liver weight with macroscopic and microscopic liver changes and increased severity of brain vacuolation occurred at 1000 ppm; 10 ppm was considered to be the no observed adverse effect level. Treatment levels of 0, 3000, 5000, or 7000 ppm in the diet were given to mice in the 4-week study (MRID 44807211). Treatment-related changes were seen in the liver of mice at all treatment levels and kidney effects were seen in males at 5000 and 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the brain were seen at four weeks in males at 3000, 5000, and 7000 ppm and in females at 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the spinal cord were also seen in males at 5000 and 7000 ppm. The LOAEL for vacuolation of white matter in the brain of male mice in this four-week study was 3000 ppm (555 mg/kg/day; lowest dose level tested). A NOAEL was not demonstrated for this effect (i.e. NOAEL <555 mg/kg/day). A more detailed summary of the 4-week study is provided in Appendix 1. Based on the results of the 4-week study, a dietary concentration of 7000 ppm, which would achieve a dose of about 1 g/kg/day, was selected as the high-dose level for this study.

4. Diet preparation and analysis

Each week an appropriate amount of ground Fluazinam was mixed with the powdered SDS Rat and Mouse No. 1 modified maintenance diet in a Turbula mixer for at least 5 minutes to prepare a pre-mix. The dietary concentrations used in the study were prepared by dilution of the pre-mix with untreated diet and mixing in a double cone blender for at least 7 minutes. Prepared diets were stored at ambient temperature in the animal room. Prior to the study initiation, samples from the 1000 and 7000 ppm mixes were taken from the top, middle, and bottom of the diet mix and 2 samples were taken at random to test for homogeneity. Samples of these diet mixtures were also analyzed after storage for 7 and 14 days at room temperature to test for stability. All dietary concentrations were analyzed for fluazinam content during weeks 1, 4, 13, 26, 39, 52, 65, 78, 84, 91, and 104. Additional analyses were performed on the 1000 ppm diets from weeks 91 and 95 preparations and from the 7000 ppm diets from weeks 78 and 84. The extra analyses were done as a precaution since the previous analyses had shown lower than expected concentrations. The dietary concentrations of fluazinam were measured using high performance liquid chromatography.

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Results

Homogeneity – The average concentrations of duplicate samples of the test material in the 1000 ppm mixture taken from the top, middle, bottom of the container and at random ranged from 98.9 to 99.6% of the target concentration for the 1000 ppm concentration and from 103.2 to 104.8% of the target concentration for the 7000 ppm concentration. .

Stability – The analysis of dietary mixtures containing 1000 ppm Fluazinam stored for 14 days at room temperature showed no decrease in concentration; the 7000 ppm mixtures contained 7171 ppm compared to a mean of 7321 at day 0.

Concentration analysis – The mean concentration at each time point ranged from 96-107% of the target concentration at 1000 ppm, 91-106% at 3000 ppm, and 88-108% at 7000 ppm. The overall mean concentration analyses showed agreement within 99±4%, 101±5%, and 99±6% of the nominal concentration for the 1000, 3000, and 7000 ppm concentrations, respectively.

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

5. Statistics

Histopathology findings were analyzed using the one-tailed Fisher's exact test. Log rank methods of Mantel and Peto were used to analyze mortality. Food consumption, body weight, hematology, organ weight, and selected pathology data were subjected to Bartlett's test for heterogeneity of variance. If significant heterogeneity of variance was found, an analysis of ranks by the Kruskal and Wallis test was used followed by non-parametric students t test or William's test. If heterogeneity of variance between treatments was not found, a one-way analysis of variance was done followed by student's t test and/or William's test for a dose-related response. For the analysis of absolute organ weights, the analysis of variance was performed using the terminal body weight as covariate. The hepatocellular tumors were analyzed using the time-to-tumor method of Peto according to the recommendations of the International Agency for Research on Cancer. Trend tests were also used and were based on the target dose levels.

Statistical significance was flagged at $p<0.05$.

C. METHODS

1. Observations

Animals were inspected twice daily during the work week and once daily on weekends and holidays for mortality and at least once daily for signs of toxicity. Each animal was given a detailed weekly examination and palpation for masses.

2. Body weight

Animals were weighed when they were allocated to groups, 2 weeks prior to treatment initiation, and at weekly intervals during the treatment period.

3. Food consumption and compound intake

Food consumption for each animal 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, the authors calculated food conversion ratios [food consumed (g)/bodyweight gain (g)], which vary inversely as the food efficiency. The compound intake (mg/kg/day) was calculated for each concentration from the food intake and body weight data.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations are not required and were not performed.

5. Blood was collected from all surviving mice during treatment weeks 52 and 78 and at study termination (week 97 for females, 104 for males). Blood samples were obtained from the orbital sinus of mice in other studies by this laboratory on Fluazinam (MRID nos. 44807211, 42208405), but the methodology for sample taking was not specified in this study. Blood samples were also taken from all mice that died at unscheduled times during the study whenever possible. Blood smears were prepared from all samples. The CHECKED (X) parameters were examined.

a. Hematology

	Hematocrit (HCT)	X	Leukocyte differential count*
	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
	Leukocyte count (WBC)		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
	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.

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b. Clinical chemistry

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

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. The animals reserved for the satellite groups were killed after 78 weeks of treatment; the terminal kill for females was during week 97 and males were terminated after 104 weeks of treatment. The satellite group at 78 weeks was immediately subjected to histopathological examination of the livers. 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 for fat 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, kidneys, and any macroscopically abnormal tissue from all animals in all groups were examined microscopically, and other indicated tissues from the control and high-dose groups only were examined microscopically. Any tissue showing a treatment-related change at the high dose was examined in all dose groups. All indicated 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 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*+	XX	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	X	All gross lesions and masses*
	Larynx	X	Vagina		

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

II. RESULTS

A. OBSERVATIONS

1. Toxicity

An increased number of animals showing the appearance of periorbital hair loss around both eyes compared to the control groups was seen at 7000 ppm in both sexes and at 3000 ppm in females from treatment week 6 to study termination, and was most frequently observed in males between weeks 53 and 78 (control, 5%; 3000 ppm, 15%; 7000 ppm 63%) and in females between weeks 79 and 97 (control, 18%; 3000 ppm, 51%; 7000, 73%). It was later found that the observation of hair loss may have been related to inversion of the eyelids, which gave the apparence of hair loss. The identity of the sign is questionable, but the observation appears to be treatment-related. There were no other treatment-related increases in the incidences of clinical observations in treated animals compared to the control groups.

2. Mortality

The percent survival at selected times during the main study is given in Table 2. Treatment-related increased mortalities occurred in the 7000 ppm female group late in the study (after 85 weeks). The overall survival of females at 7000 was significantly decreased compared to the control group (control, 58%; 7000 ppm, 26%, $p<0.01$). All

females in the study were terminated at week 97 because of the increased mortality in the high-dose group. No treatment-related decreases in survival were seen in males, and the males continued in the study through 104 weeks. The survival at study termination in high-dose males was 32% compared to 36% in the controls. At term, the numbers of survivors for males (at 104 weeks) was 18, 26, 21 and 16 and for females (at 97 weeks) was 29, 26, 27 and 13 for the control, 1000 ppm, 3000 ppm and 7000 ppm groups respectively.

Atrial thrombus was listed as a factor contributing to the unscheduled death of about 19% and 9% of control males and females, and 46% and 30% of high-dose males and females, respectively. Vacuolation of white matter in the central nervous system contributed to the unscheduled death of about 9% of high-dose males and 19% of high-dose females and did not contribute to the death of any control animals. No other factors were predominate in the unscheduled deaths of treated mice compared to the control groups.

TABLE 2. Percent survival of male and female mice fed Fluazinam for up to 104 weeks

Study interval	Dietary concentration (ppm)			
	0	1000	3000	7000
Males (n=50)				
Weeks 1-52	96	96	94	88
Weeks 1-78	74	76	80	80
Weeks 1-104	36	52	42	32
Females (n=50)				
Weeks 1-52	96	92	96	98
Weeks 1-78	78	66	68	64
Weeks 1-97	58	52	54	26**

Data taken from Table 1, pp. 54-57, MRID 44807222

**$p<0.01$, Significantly different from controls.

B. BODY WEIGHT

Selected group mean body weights and body weight gains in male and female mice during treatment are summarized in Table 3. The overall group mean body weights of high-dose males were slightly lower than the control weights throughout most of the study (from about week 4 to week 97), but the difference was not statistically significant. The body weights of high-dose females were comparable or slightly higher than that of the control group throughout most of the study. The group mean body weight gain measured between weeks 4 to 36 was decreased in high-dose males by about 32% compared to the controls ($p<0.01$). The overall cumulative weight gain of males at week 104 was about 10% (NS) less at 7000 ppm than in the control group. The body weight gain in high-dose females was not significantly decreased compared to the controls.

TABLE 3. Group mean body weight and body weight gains in male and female mice fed Fluazinam for up to 104 weeks (g)

Body weight or weight gain measured on test day	Dietary concentration (ppm)			
	0	1000	3000	7000
Males				
Body wt. at week 0	29	29	29	30
Body wt. at week 36	46	48	45	42
Body wt. at week 104	43	45	43	43
Wt. gain week 4-36	11.2 ± 3.98[a]	12.0 ± 5.09	10.0 ± 3.85	7.6 ± 2.58**(-32%)[b]
Wt. gain week 0-97	16.3 ± 5.70	17.5 ± 7.34	16.4 ± 4.99	13.4 ± 4.84
Wt. gain week 0-104	14.5 ± 5.83	16.2 ± 5.94	14.2 ± 5.06	13.1 ± 5.09(-10%)
Females				
Body wt. at week 0	23	23	23	23
Body wt. at week 36	34	35	35	34
Body wt. at week 97	39	40	38	38
Wt. gain week 4-36	7.9 ± 4.59	7.8 ± 4.30	7.6 ± 4.36	7.3 ± 3.43
Wt. gain week 0-97	15.6 ± 6.35	16.0 ± 5.47	15.1 ± 4.50	14.4 ± 4.56

Data taken from Table 2 pp. 58-61, and p. 30, MRID 44807222.
[a]Mean ± Standard deviation
[b]Numbers in parentheses are the percent change compared to the control group.
**$p<0.01$, Significantly different from controls.

C. FOOD CONSUMPTION AND COMPOUND INTAKE

1. Food consumption

Selected food consumption values are summarized in Table 4. No treatment-related changes were seen in food consumption in either sex; high-dose females consumed slightly more food (~6% NS) over the course of the study than the control group.

TABLE 4. Group mean weekly food consumption and food conversion ratios in male and female mice fed Fluazinam for up to 104 weeks (g/mouse)

Food consumption and efficiency study period (weeks)	Dietary concentration (ppm)			
	0	1000	3000	7000
Males				
Food consumption/ wks. 1-97	3769 ± 308.3[a]	3882 ± 302.3	3688 ± 381.7	3736 ± 273.9
Food consumption/ wks. 1-104	4081 ± 333.3	4202 ± 344.2	3965 ± 459.7	4033 ± 296.1
Food conversion ratio/ wks. 9-13	77.3	109.7	137.0 (+77%)[b]	143.7 (+86%)
Food conversion ratio/ wks. 1-13	49.1	48.0	52.5 (+7%)	64.0 (+30%)
Food efficiency/ days 0-104[c]	0.355	0.386	0.358	0.325 (-8%)
Females				
Food consumption/ wks. 1-97	3712 ± 344.7	3816 ± 312.7	3637 ± 406.4	3933 ± 270.3
Food conversion ratio/ wks. 9-13	200.8	135.0	98.7	151.0
Food conversion ratio/ wks. 1-13	81.9	74.8	69.1	79.8
Food efficiency/ days 0-97[c]	0.420	0.419	0.415	0.366 (-13%)

Data taken from pp. 30-31 and Table 4, p. 66, MRID 44807222
[a]Mean ± standard deviation
[b]Numbers in parentheses are the percent difference from the control group.
[c]Food efficiency (g weight gained/g food consumed X 100) was calculated by the reviewer from the overall weight gain and food consumption.

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 is summarized in Table 4. Food conversion ratios (food consumed/bodyweight gain in grams) were calculated by the study authors over the first 13 weeks of the study corresponding to a rapid growth period of the mice. The ratios were increased in males by 77% and 86% at 3000 and 7000 ppm, respectively, for treatment weeks 9-13 and by 7% and 30% over the entire first 13-week period compared to the control group. The food conversion ratios for females were not increased over the first 13 weeks of the study. The overall food efficiency for the entire study, which varies inversely as the food conversion ratios, was estimated by the reviewer from the body weight changes and the food intake measurements. The food efficiency was decreased by about 8% in males, and by 13% in females at 7000 ppm compared to the control group.

D. BLOOD WORK

1. Hematology

The differential counts did not reveal any treatment-related changes in the white blood cell distributions in treated animals compared to the controls. Some differences between the high dose animals and the controls were observed that were statistically significant, but the changes were not consistent from one time point to another, did not show a clear dose effect, and were not seen in both sexes.

E. SACRIFICE AND PATHOLOGY

1. Organ weight

The final body weights and selected absolute organ weights are summarized in Table 5. The group mean adjusted absolute liver weights of high-dose males were increased by 65% ($p<0.01$) compared to the controls after 78 weeks of treatment and by 182% ($p<0.01$) after 104 weeks. The adjusted liver weights were also increased by 54% and 113% at 1000 and 3000 ppm, respectively, after 104 weeks compared to the control group. The group mean adjusted absolute liver weights of females were also increased by about 90% at 7000 ppm after 78 weeks and by 21%, 45%, and 109% at 1000, 3000, and 7000 ppm, respectively, compared to the control group after 97 weeks of treatment ($p<0.01$). The absolute liver weights were adjusted for the final body weights in the statistical analysis to compensate for changes attributable to body size. The adjusted absolute brain weights were increased in high-dose males by 8% ($p< 0.01$) after 104 weeks of treatment and by 7% ($p<0.01$) in high-dose females after 97 weeks of treatment. Absolute adrenal weights were slightly increased in high dose males by about 25% (NS) and by 18% ($p<0.05$) in females. Absolute spleen weights were increased in treated males by 37-50% ($p<0.05$) and in treated females by 5-78% (NS), but the increases were not dose-related in either sex and not statistically significant in females.

TABLE 5. Group mean organ and final body weights in male and female mice fed Fluazinam for up to 104 weeks (grams)				
Organ	Dietary concentration (ppm)			
	0	1000	3000	7000
Males				
Final body, 78 weeks	45.3	–	–	42.5
Final body, 104 weeks	43.0	44.7	43.1	42.3
Liver, 78 weeks[a]	2.44	–	–	4.02**(+65%)
Liver, 104 weeks[a]	2.09	3.22**(+54%)[b]	4.46**(+113%)	5.90**(+182%)
Brain, 104 weeks[a]	0.485	0.482	0.487	0.524**(+8%)
Spleen, 104 weeks	0.086	0.129*(+50%)	0.118*(+37%)	0.124*(+44%)
Adrenal, 104 weeks	0.0063	0.0065	0.0073	0.0079(+25%)
Females				
Final body, 78 weeks	35.7	–	–	37.3
Final body, 97 weeks	37.9	39.0	38.0	37.5
Liver, 78 weeks[a]	1.67	–	–	3.17**(+90%)
Liver, 97 weeks[a]	1.84	2.23**(+21%)	2.67**(+45%)	3.85**(+109%)
Brain, 97 weeks	0.507	0.491	0.500	0.544**(+7%)
Spleen, 97 weeks	0.121	0.127(+5%)	0.215(+78%)	0.175(+45%)
Adrenal, 97 weeks	0.0101	0.0093	0.0099	0.0119*(+18%)

Data taken from Table 7, pp. 75-77, MRID 44807222
[a]Liver weight and male brain weight were adjusted for changes in final body weights.
[b]Numbers in parentheses are the percent difference from control group.
*p<0.05, **p<0.01, Significantly different from the control.

2. Gross pathology

Selected macroscopic findings from the main study are summarized in Table 6. Increased incidences of enlarged liver were seen in both sexes at 3000 and 7000 ppm compared to the controls (p<0.01, except for females at 3000 ppm, p<0.05). The incidences of livers with pale areas were increased in males at 3000 and 7000 ppm (p<0.01) and in females at 7000 ppm (p<0.01). The number of mice in which most of the entire liver was pale increased in high-dose males (control, 14/50, 7000 ppm, 27/50, p<0.01) and in females at 3000 and 7000 ppm (control, 11/50, 3000 ppm, 20/50, p<0.01; 7000 ppm, 21/50, p<0.01). Incidences of accentuated lobular markings were increased in the livers of males especially at 7000 ppm (p<0.01), but the incidences did not show a clear dose effect in males. In females the accentuated lobular markings were increased at 3000 and 7000 ppm compared to the control and

were dose-related ($p<0.01$). Slightly increased incidences of masses in the liver of males and females were found at both 3000 ppm ($p<0.05$) and at 7000 ppm (males: 23/50, $p < 0.01$, females: 4/50, NS) compared to the controls 13/50 and 1/50 for males and females, respectively.

Incidences of thrombi in the left atrium of the heart were increased in males and females at 7000 ppm (males, $p<0.05$; females, $p<0.01$). The atrial thrombi were primarily associated with animals that died during the course of the study. Incidences of mice with pale kidneys decreased in treated males ($p<0.01$-0.05) and increased in treated females ($p<0.05$). Swelling of the brain was noted in 2 males and 3 females at 7000 ppm and in 1 female at 3000 ppm.

TABLE 6. Macroscopic findings in male and female mice fed Fluazinam for up to 104 weeks (no. of mice with lesion)

Organ or tissue/lesion	Dietary concentration (ppm)			
	0	1000	3000	7000
Males (n = 50)				
Liver/ enlarged	3	6	20**	27**
Liver/ pale areas	7	12	20**	30**
Liver/ pale	14	11	14	27**
Liver/ lobular markings accentuated	3	11*	9	15**
Liver/ masses	13	18	25*	23*
Heart/ thrombus in left atrium	7	9	15	17*
Kidney/ pale	19	11	8*	6**
Brain/swelling	0	0	0	2
Female (n = 50)				
Liver/ enlarged	2	0	8*	14**
Liver/ pale areas	3	4	8	17**
Liver/ pale	11	11	20**	21**
Liver/ lobular markings accentuated	0	3	11**	18**
Liver/ masses	1	1	6*	4
Heart/ thrombus in left atrium	1	8	4	11**
Kidney/ pale	7	18*	16*	16*
Brain/swelling	0	0	1	3

Data taken from Table 8, pp. 87-98, MRID 44807222

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

3. Microscopic pathology

a. Non-neoplastic

Selected non-neoplastic microscopic findings in the main study after treatment for up to 104 weeks in males and up to 97 weeks in females are summarized in Table 7. The findings in mice that died at unscheduled times during the study and the terminal findings were combined in the table. The incidences of hepatocyte enlargement, hepatocytes with a pale or vacuolated cytoplasm, and aggregates of brown pigmented macrophages in the liver were significantly ($p<0.01$) increased in all treated animals compared to the controls. The severity of the brown pigmented macrophage aggregates also increased with increasing concentrations of fluazinam from none of the lesions in control males showing "moderate" or "marked" severity to 58% at 7000 ppm ($p<0.01$) and 22% of lesions in female controls to 41% in high-dose females ($p<0.01$). Incidences of altered hepatocyte foci, which include basophilic and eosinophilic vacuolated cells, clear cells, and altered ORO stained (for fats) cells, were significantly ($p<0.05$ or 0.01) increased at all doses in males and in females at 7000 ppm ($p<0.01$). Incidences of brown pigmented centrilobular hepatocytes and parenchymal inflammatory cells graded minimum or "moderate" were increased in males at 3000 and 7000 ppm compared to the controls ($p< 0.05$) but were not significantly increased in females.

The incidences of vacuolation of white matter in the brain were increased in all treated animals and were also increased in the spinal cord at 3000 ppm in males and in high-dose males and females. White matter vacuolation was seen in the cerebrum of 41/50 males at 1000 ppm, 50/50 males at 3000 ppm and in 49/50 at 7000 ppm ($p<0.01$) compared to 24/50 in the control group. In females, the incidences of white matter vacuolation in the cerebrum were 39/50 in the control, 45/50 (NS) at 1000 ppm, 49/50 at 3000 ppm, and 50/50 at 7000 ppm ($p<0.01$). Similar increases in vacuolation of white matter were seen in the cerebellum, pons, and medulla of treated animals compared to controls (controls: 24 and 43/50; 1000 ppm, 40 and 44/50; 3000 ppm, 45 and 49/50; 7000 ppm, 48 and 49/50, $p<0.01$ for males and females, respectively). Incidences of white matter vacuolation in the spinal cord were slightly increased in males at 1000 ppm (24/50, NS) compared to the control group (18/50) and significantly increased at 3000 ppm (37/50, $p<0.05$) and 7000 ppm (46/50, $p<0.01$), and were slightly increased in females at 3000 ppm and 7000 ppm (45/50, NS). The severity of the vacuolation of white matter in the brain and spinal cord markedly increased with increasing concentration of fluazinam. No lesions in the brain or spinal cord in the male or female control groups were graded "moderate" or "marked" and only 0-5% were so graded at 1000 ppm. In contrast, 12-27% of brain lesions at 3000 ppm and 42-60% of brain lesions at 7000 were graded "moderate" or "marked" ($p<0.05$ or 0.01). Vacuolation of white matter in the spinal cord was graded "moderate" or "marked" in 33% of males and in 47% of females that had this lesion ($p<0.01$) at 7000 ppm.

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Incidences of thrombi in the left atrium of the heart were increased in high-dose males ($p<0.05$) and females ($p<0.01$). The incidences of amyloidosis in the heart increased slightly, but not significantly, in high-dose animals; however, the severity increased from about 29% of the lesions graded "marked" in male controls and 21% in females to 68% ($p<0.05$) and 53% (NS) of the lesions in high-dose males and females, respectively. The left atrial thrombi were usually associated with "moderate" or "marked" amyloidosis and the lesions were seen mostly in animals that died at unscheduled times during the study.

The histopathological findings in liver, brain, and heart in the 78-week satellite study involving 20 males and 18 females fed diets containing 0 or 7000 ppm fluazinam were similar to those of the main study, but the incidences were lower and did not reach statistical significance in some cases (atrial thrombus). Incidences of submucosal mononuclear cells in the glandular region of the stomach were increased in both sexes at 7000 ppm compared to the control groups after 78 weeks (males: control, 0/20; 7000 ppm, 7/20, $p<0.01$; females: control, 0/20; 7000 ppm, 7/20, $p<0.05$), but were not increased in high-dose mice at the study termination.

TABLE 7. Non-neoplastic histopathology findings in male and female mice fed Fluazinam for up to 104 weeks

Organ/tissue/finding	Dietary concentration (ppm)			
	0	1000	3000	7000
Males (n = 50)				
Liver/ altered hepatocyte foci	12	24*	36**	33**
Liver/ hepatocyte enlargement	9	35**	34**	41**
Liver/ pale or vacuolated hepatocyte cytoplasm	0	17**	31**	39**
Liver/ brown pigmented centrilobular hepatocytes	0	3	6*	11**
Liver/ parenchymal inflammatory cells (min. or mod.)	1	5	6*	16**
Liver/ brown pigmented macrophages, aggregates	5(0)[a]	30**(17)	41**(24**)	45**(58**)
Brain, cerebrum/ vacuolation of white matter	24(0)[a]	41**(0)	50**(12*)	49**(45**)
Brain, cerebellum, pons, medulla/ vacuolation of white matter	24(0)[a]	40**(3)	45**(16*)	48**(42**)
Spinal cord/ vacuolation of white matter	18(0)[a]	24(0)	37*(3)	46**(33**)
Heart/ atrial thrombus	7	8/26[b]	16**/30	18*
Heart/ amyloidosis	14(29)[c]	14/26[b](21)	12/30(75*)	19(68*)
Glandular stomach/ epithelial hyperplasia	11	6	12	11

TABLE 7. Non-neoplastic histopathology findings in male and female mice fed Fluazinam for up to 104 weeks				
Females (n=50)				
Liver/ altered hepatocyte foci	3	4	7	15**
Liver/ hepatocyte enlargement	0	18**	35**	28**
Liver/ pale or vacuolated hepatocyte cytoplasm	0	11**	24**	27**
Liver/ brown pigmented centrilobular hepatocytes	0	0	0	1
Liver/ parenchymal inflammatory cells (min. or mod.)	1	0	2	5
Liver/ brown pigmented macrophages, aggregates	9(22)[a]	27**(4)	41**(29*)	41**(41**)
Brain, cerebrum/ vacuolation of white matter	39(0)[a]	45(2)	49**(24**)	50**(60**)
Brain, cerebellum, pons, medulla/ vacuolation of white matter	43(0)[a]	44**(5)	49**(27**)	49**(57**)
Spinal cord/ vacuolation of white matter	37(0)[a]	36(0)	45(7)	45(47**)
Heart/ atrial thrombus	2	9**/24[b]	3/23	12**
Heart/ amyloidosis	14(21)[c]	12/24[b](25)	12/23(8)	17(53)
Glandular stomach/ epithelial hyperplasia	8	9	8	17*

Data taken from pp. 38-44 and Table 9, pp. 167-262, MRID 44807222
[a]Numbers in parentheses are percent of lesions graded "moderate" or "marked."
[b]/Total number of mice if less than 50
[c]Numbers in parentheses are percent of lesions graded "marked" in the case of cardiac amyloidosis.
*p<0.05, **p<0.01, Significantly different from controls, Fisher exact test by reviewer.

b. Neoplastic

IMPORTANT COMMENTS

In an addendum report (MRID 44807221, dated 8/28/98) to the main report for this study (MRID 44807222, dated 12/19/96), the histopathological diagnoses for 3 mice in this study were changed. The following is quoted from page 6 of the addendum report.

> "In May 1998, at the request of the Sponsor, the report (Huntingdon Life Sciences Report No. ISK 50/950671) was reviewed, and, following a discussion between the Sponsors and Huntingdon Life Sciences, some emphasis changes were suggested. Slides from male mice showing hepatocellular tumours and other lesions reported macroscopically as liver masses were also reviewed on this occasion. As a result of this meeting, a further peer review of a small number of slides was undertaken by Dr. Gopinath [the original study pathologist], and in the light of this review, the diagnosis for three of the animals was changed.
>
> Mouse 3 (Control) New diagnosis Hepatocellular adenoma
> *Original diagnosis* *Basophiloic and vacuolated hepatocytes (Area)*

Mouse 23 (Control)	New diagnosis	Hepatocellular adenoma
	Original diagnosis	*Eosinophilic hepatocytes (Area - one of several)*
Mouse 106 (1000 ppm)	New diagnosis	Hepatocellular carcinoma
	Original diagnosis	*Hepatocellular adenoma*

The incidences of hepatocellular tumours in male mice were therefore again subjected to statistical analysis by time-to-tumour methods, the details of which are presented in the STATISTICAL ANALYSIS OF HEPATOCELLULAR TUMOURS IN MICE report on page 27 of this Addendum. At the request of the Sponsor, where any animal had both benign and malignant hepatocellular tumours, only the malignant tumour was considered for statistical analysis. This affected four animals: 134 and 155 (3000 ppm), and 196 and 213 (7000 ppm). Benign and malignant tumours were analysed separately.

As a result of this review and statistical analysis, the Summary, the Microscopic Pathology section, and the Discussion and Conclusion have been amended, and these amended sections are presented in full in this addendum."

With respect to the acceptability of this addendum report, HED has determined that Pesticide Registration (PR) Notice 94-5 (pertaining to changes in pathology diagnoses of microscopic slides) is applicable to this addendum report. This determination was made in an ad hoc meeting of HED toxicologists (including 2 members of the HED Cancer Assessment Review Committee) on 3/29/00. PR Notice 94-5, which took effect in 1994, specifies precise procedures to be followed when re-evaluations of microscopic slides are made. The procedures require that all slides containing the target tissue (liver, in this case) in all dose groups, as well as the controls, be re-read by a peer review pathologist and compared to the original readings by the study pathologist. A Pathology Working Group (PWG), consisting of a chair, the study pathologist, the peer review pathologist and additional consultant pathologists, must then be convened to review, as a minimum, all slides for which there were significantly differing diagnoses between the study and peer review pathologists. A copy of PR Notice 94-5, containing additional more detailed information on the procedures to be followed, is attached to this DER (Appendix 2).

Therefore, since the procedures used to change the histopathological diagnoses for 3 mice in this study were not performed in accordance with PR Notice 94-5, HED considers all information based on the re-evaluation of the slides in this addendum to be unacceptable . HED will use the information in the original 1996 study report (including the statistical analyses, the Summary, the Microscopic Pathology Section and the Discussion and Conclusion) in its evaluation of this study. Additional historical control data for liver tumors for the years 1991-1993, provided in the addendum report on page 13, however, were considered to be acceptable and were used to assist in the evaluation of the neoplastic findings in this study.

Should the Sponsor, at some time in the future, provide HED with changed histopathological diagnoses for the mice in this study performed in accordance with PR Notice 94-5, HED will reconsider this determination.

b. Neoplastic (continued)

A summary of neoplasms seen in this study is given in Table 8. The incidences of hepatocellular adenoma were significantly increased in males at 3000 ppm and 7000 ppm (control, 6/50; 1000 ppm, 13/50, NS; 3000 ppm, 22/50, $p<0.01$; 7000 ppm, 16/50, $p<0.01$). A one-tailed trend test (using groups with dose 0 up to the dose level for that row) indicated a statistically significant ($p<0.01$) positive trend at 3000 and 7000 ppm. The hepatocellular carcinoma incidence increased from 1/50 in the control to 2/50 at 1000 ppm, 3/50 at 3000 ppm and 4/50 at 7000 ppm, but the increases were not statistically significant. The trend test also was not statistically significant for any dose level. The number of males with hepatocellular adenoma and/or carcinoma increased from 7/50 in the control to 15/50 (NS) at 1000 ppm, 23/50 at 3000 ppm ($p<0.01$) and 18/50 ($p<0.01$) at 7000 ppm. The one-tailed trend test indicated a statistically significant ($p<0.01$) positive trend at 3000 and 7000 ppm. The incidences of hepatocellular adenomas in female mice were slightly higher at 3000 and 7000 ppm (3/50) than in the control group (1/50), but were not significantly different statistically from the control. The trend test also was not statistically significant for any dose level. Combining the adenoma and carcinoma incidences in females did not result in a significant difference between the treated mice and the control group and the trend test was not statistically significant for any dose level.

The total numbers of tumor bearing mice, of mice with benign tumors and of mice with malignant tumors were similar in the treated and control groups.

Incidences of lung adenomas and/or adenocarcinomas were not statistically significantly increased in treated male or female mice in the main group in this study. Also, incidences of uterine tumors were not increased in the treated main group female mice in this study. In addition, there were no significant changes in the incidences of tumors in the 78-week satellite study.

TABLE 8. Neoplastic findings in male and female mice fed Fluazinam for up to 104 weeks				
Organ and/or neoplasm	Dietary concentration (ppm)			
	0	1000	3000	7000
Males (n=50)				
Liver/ hepatocellular adenoma	6 (12 %)(a)	13 (26 %)	22**## (44 %)	16**## (32 %)
Liver/ hepatocellular carcinoma	1 (2 %)	2 (4 %)	3 (6 %)	4 (8 %)
Liver/ hepatocellular adenoma and/or carcin.	7 (14 %)	15 (30 %)	23**##(b) (46 %)	18**##(b) (36 %)
Total no. mice with tumors	31	35	41	33
Total no. mice with benign tumors	23	29	33	26
Total no. mice with malignant tumors	12	18	18	13
Females (n=50)				
Liver/ hepatocellular adenoma	1 (2 %)	0 (0 %)	3 (6 %)	3 (6 %)
Liver/ hepatocellular carcinoma	0 (0 %)	0 (0 %)	1 (2 %)	0 (0 %)
Liver/ hepatocellular adenoma and/or carcin.	1 (2 %)	0 (0 %)	4 (8 %)	3 (6 %)
Total no. mice with tumors	27	20	27	26
Total no. mice with benign tumors	14	13	17	10
Total no. mice with malignant tumors	15	7	18	18

Data taken from p. 36, pp. 2221-2222 and Table 9, pp. 158-166, MRID 44807222.
(a) Percentage incidence in parentheses
(b) Two mice had both an adenoma and a carcinoma
**$p<0.01$, one-tailed pairwise comparisons against the control group
$p<0.01$, one-tailed trend test using groups with dose 0 up to the dose level for that row

Historical control data for liver cell tumors in male and female mice were provided in the study report for 9 studies started in the same testing laboratory in 1986-1988 with study durations of 92-104 weeks. See Table 9. For males, the range for hepatocellular adenomas was 8-23% and for hepatocellular carcinomas was 5-13%. For females, the range for hepatocellular adenomas was 0-5% and for hepatocellular carcinomas was 0-2%.

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TABLE 9. Historical control data for liver cell tumors in male and female mice 1986-1988; 92-104 weeks (Percent incidence)

Organ / neoplasm	Study number								
	1	2	3	4	5	6	7	8	9
Males									
Liver/hepatocellular adenoma	10	8	23	9	11	15	10	9	13
Liver/hepatocellular carcinoma	6	6	9	11	9	13	8	5	5
Females									
Liver/hepatocellular adenoma	0	2	5	0	0	0	4	0	0
Liver/hepatocellular carcinoma	0	0	0	0	2	0	0	2	0

Data taken from p. 2207, MRID 44807222.

Additional historical control data for liver cell tumors in male and female mice were also provided in the addendum report (MRID 44807221, dated 8/28/98) for 12 studies started in the same testing laboratory in 1991-1993 with study durations of 80-96 weeks. See Table 10. For males, the range for hepatocellular adenomas was 8-34% (the next highest percent was 22%) and for hepatocellular carcinomas was 2-16%. For females, the range for hepatocellular adenomas was 0-4% and for hepatocellular carcinomas was 0-0%.

TABLE 10. Additional historical control data for liver cell tumors in male and female mice 1991-1993; 80-96 weeks (Percent incidence)

Organ / neoplasm	Study number											
	1	2	3	4	5	6	7	8	9	10	11	12
Males												
Liver/ hc adenoma	8	11	20	8	16	14	16	22	14	12	16	34
Liver/ hc carcin.	8	14	9	10	2	12	6	4	4	12	6	16
Females												
Liver/hc adenoma	0	0	0	0	0	0	2	4	0	0	0	2
Liver/hc carcin.	0	0	0	0	0	0	0	0	0	0	0	0

Data taken from p.13, MRID 44807221.

Historical control data for combined hepatocellular adenomas and/or carcinomas were not provided in the study report.

For male mice in this study, the percentage incidence of hepatocellular adenomas at 3000 ppm (44%) exceeded the highest value for historical control data for studies with durations of 92-104 weeks (23%) and for studies with durations of 80-96 weeks (34%). The percentage incidence at 7000 ppm (32%) also exceed the highest historical control value for 92-104 week studies (23%), but not for 80-96 week studies (34%). It is noted that for all 21 historical control studies, the next highest percentage incidence value for hepatocellular adenomas, excluding the single study with a percentage incidence of 34%, is 23%. For male mice, the highest percentage incidence of hepatocellular carcinomas (8% at 7000 ppm) did not exceed the highest value for historical control data for any of the 21 historical control studies (16%).

For female mice in this study, the percentage incidence of hepatocellular adenomas at 3000 ppm and 7000 ppm (both 6%) slightly exceeded the highest value for historical control data for studies with durations of 92-104 weeks (5%) and for studies with durations of 80-96 weeks (4%). For female mice, the highest percentage incidence of hepatocellular carcinomas (2% at 3000 ppm) did not exceed the highest value for historical control data for any of the 21 historical control studies (2%).

The increased incidence of hepatocellular adenomas and of combined hepatocellular adenomas and/or carcinomas observed in male mice at 3000 ppm and 7000 ppm are considered to be treatment-related.

III. DISCUSSION

A. INVESTIGATOR'S CONCLUSIONS

The investigators concluded that the following treatment-related effects were observed in mice treated with Fluazinam in this study.

At 7000 ppm: increased mortality in males; increased (apparent) periorbital hair loss around both eyes in males and females; decreased body weight gain (during weeks 4-36) in males; decreased efficiency of food utilization in males; increased absolute and liver/body weight ratios in males and females; increased brain weights in males and females; increased gross findings, particularly increased pathologic findings in the liver of males and females and also increased swelling of the brain in 2 males and 3 females; increased histopathologic findings, particularly increased findings in the liver of many males and females and vacuolation of white matter in the brain and cervical spinal cord in many males and females; and a treatment-related increased incidence of hepatocellular adenomas and of combined adenomas and/or carcinomas in the livers of male mice. In the addendum report (MRID 44807221), based on revised histopathological diagnoses for 3 mice, the hepatocellular tumors observed in male mice at this dose level were re-evaluated, were considered to be within the historical control range of data at the performing laboratory, and were considered to not be treatment-related. [Because this re-

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evaluation was not performed in accordance with PR Notice 94-5, however, this re-evaluation was not acceptable to EPA]. Treatment-related tumors were not observed in the female mice at this dose level.

At 3000 ppm: increased (apparent) periorbital hair loss around both eyes in females; increased absolute and liver/body weight ratios in males and females; increased gross findings, particularly increased pathologic findings in the livers of males and females and also increased swelling of the brain in 1 female; increased histopathologic findings, particularly increased findings in the livers of many males and females and vacuolation of white matter in the brain and cervical spinal cord in many males and females; and a treatment-related increased incidence of hepatocellular adenomas and of combined adenomas and/or carcinomas in the livers of male mice. In the addendum report (MRID 44807221), based on revised histopathological diagnoses for 3 mice, the hepatocellular adenomas observed in male mice at this dose level were still considered to be treatment-related due to a statistically significant pairwise difference with the control group and a positive trend test. Statistical analyses were not presented in the addendum report for combined hepatocellular adenomas and/or carcinomas. Treatment-related tumors were not observed in the female mice at this dose level.

At 1000 ppm: increased absolute and liver/body weight ratios in males and females; increased gross findings in the livers of some males; increased histopathologic findings in the livers of many males and females; and vacuolation of white matter in the brain and cervical spinal cord in 1 male and 2 females (considered to be a marginal effect at this dose). A treatment-related increased incidence of tumors was not observed in either the male or female mice at this dose level.

B. REVIEWER'S DISCUSSION

The only clinical sign that was observed during the study was the appearance of periorbital hair loss seen at 7000 ppm in both sexes and at 3000 ppm in females. The sign was most frequently seen in males between weeks 53 and 78 when the observation frequency of the sign was increased from 5% in the controls to 63% at 7000 ppm and in females between weeks 79 and 97 and seen in 18% of controls and 73% of females at 7000 ppm. It was later discovered that some animals experienced inversion of the eyelids, which gave the appearance of periorbital hair loss making the nature of the sign questionable. Nevertheless, the observation appeared to be treatment-related.

All female groups in the study were terminated after 97 weeks of treatment because of decreased survival in the 7000 ppm group (control, 58%; 7000 ppm, 26%, $p<0.01$). The survival of males at 7000 ppm was similar to that of the control group; therefore, they were allowed to continue in the study for the full 104 weeks (control at 104 weeks, 36%; 7000 ppm, 32%). The group mean body weights of high-dose males were slightly decreased and high-dose females were slightly increased at most weeks in the study, but the differences were not statistically significant. The body weight gain measured for treatment weeks 4-36 was decreased 32% ($p<0.01$) compared to the control in males at

7000 ppm. No treatment-related changes were seen in food consumption in either sex. Food conversion ratios calculated for males over weeks 9-13 were about 86% higher at 7000 ppm than the control group and were 30% higher over weeks 1-13. These values reflect the decrease in body weight gain during this period and are indicative of a decreased efficiency of food utilization during these time periods in males. The overall food efficiency for the study was decreased by 8% in males and by 13% in females at 7000 ppm compared to the controls, which is consistent with a toxic response at the high dose.

There were no treatment-related changes in the differential white cell count or morphology at any period in the study. No other hematological parameters were measured in the study.

The group mean adjusted absolute liver weights of males and females were significantly ($p<0.01$) increased at all dose levels compared to the control groups at study termination (males: increased by 54%, 113% and 182%; females: 21%, 45%, and 109% at 1000, 3000, and 7000 ppm, respectively). The group mean absolute brain weights (adjusted for males only) were increased in high-dose males by about 8% and in high-dose females by 7% ($p<0.01$) compared to the controls. Absolute adrenal weights were increased at 7000 ppm compared to the controls by 25% in males (NS) and 18% in females ($p<0.05$), and the absolute spleen weight was increased in high-dose males by about 44% compared to the controls ($p<0.05$). There were no gross or microscopic necropsy findings to explain or support the changes in spleen or adrenal weights in high-dose animals. However, the necropsy findings confirmed the liver and brain as the primary sites for the toxic effects of fluazinam treatment in this study. Increased incidences of enlarged liver and liver with pale areas, accentuated lobular markings, and pallor were increased in both sexes at 3000 and 7000 ppm and marginally at 1000 ppm. The incidence of liver masses were increased in mid- and high-dose males and marginally in mid-dose females. The weight changes and macroscopic findings in the liver were supported by microscopic findings. Increased incidences of mice with livers containing altered hepatocyte foci (vacuolated basophilic or eosinophilic cells, clear cells, and cells stained for fat) were seen at all concentration levels in males and in high-dose females (males: control, 12/50; 1000 ppm 24/50, $p<0.05$; 3000 ppm, 36/50, 7000 ppm, 33/50, $p<0.01$; females control, 3/50; 7000 ppm 15/50, $p<0.01$). Incidences of livers with hepatocyte enlargement, hepatocytes with a pale or vacuolated cytoplasm, and aggregates of brown pigmented macrophages were increased at all doses in males and females compared to the control incidences (controls, 0-9/50; 1000 ppm, 11-35/50; 7000 ppm 27-45/50, $p<0.01$). The aggregates of brown pigmented macrophages also increased in severity with increasing concentration. Incidences of brown pigmented centrilobular hepatocytes and liver parenchymal inflammatory cells were significantly increased in males at 3000 and 7000 ppm (control, 0-1/50; 3000 ppm, 6-6/50, $p<0.05$; 7000 ppm, 11-16/50, $p<0.01$ for pigmented hepatocytes and inflammatory cells, respectively). Males appeared more sensitive to the hepatotoxic effects of fluazinam than females.

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Microscopic examination of the central nervous systems revealed increased incidences of vacuolation of white matter in the brains of all treated animals ($p<0.01$). Vacuolation of white matter was also increased in the cervical spinal cord in males at 3000 ppm ($p<0.05$) and 7000 ppm (46/50, $p<0.01$) compared to the controls, and marginally in females at 3000 and 7000 ppm (NS) compared to the control group. Increased dietary concentrations of fluazinam resulted in increases in the severity of white matter vacuolation. No lesions were found in the controls that were graded "moderate" or "marked", whereas 33-45% of the lesions in males and 45-50% of lesions in females at 7000 ppm were so graded. Vacuolation of white matter in the central nervous system was cited by the study authors as contributing to the cause of death in 9% of males and 19% of females at 7000 ppm; compared to none in the control group.

Another lesion cited as contributing to the cause of death in 46% of high-dose males compared to 19% in the controls and 30% of high-dose females compared to 9% in the female control group was thrombi in the left atrium of the heart. The incidences of left atrial thrombus were increased in males and females at the high dose compared to the controls (males: control, 7/50; 7000 ppm, 18/50, $p<0.05$; females: control, 2/50; 7000 ppm, 12/50 $p<0.01$). The atrial thrombi were associated with amyloidosis, which increased in severity at 7000 ppm in both sexes. Amyloidosis, found commonly in aging mice, marginally increased in incidence and/or severity in many tissues examined. One of the effects of fluazinam toxicity seems to be the exacerbation of pathological conditions that normally occur in aging mice.

The Lowest-observed-adverse-effect-level (LOAEL) seen in this study was 1000 ppm (126 mg/kg/day for males, 162 mg/kg/day for females) based on increased liver weights in males and females and microscopic liver and brain changes. A no-observed-adverse-effect-level (NOAEL) was not determined in this study (<1000ppm)..

Treatment of Crl:CD®-1 mice for up to 104 weeks resulted in treatment-related increases in hepatocellular adenoma in males at 3000 ppm and 7000 ppm (controls, 6/50; 3000 ppm, 22/50; 7000 ppm 16/50, $p<0.01$). The hepatocellular carcinoma incidence was also increased in high-dose males, but was not statistically significant (control, 1/50; 7000 ppm, 4/50, NS), and was within the range of historic control incidences. The incidences of combined hepatocellular adenoma and/or carcinoma were also increased in males at 3000 ppm and 7000 ppm (control, 7/50; 3000 ppm, 23/50; 7000 ppm, 18/50, $p<0.01$). Two male mice each at 3000 and 7000 ppm were found to have both hepatocellular adenoma and carcinoma. The increased incidence of hepatocellular adenomas and of combined hepatocellular adenomas and/or carcinomas observed in male mice at 3000 ppm and 7000 ppm are considered to be treatment-related. Although the hepatocellular adenoma incidences in females at 3000 and 7000 ppm (3/50) were slightly higher than the control group (1/50), they were not statistically significant, and were only marginally higher than the upper range of incidences seen in historical control animals supplied with the study. The liver and brain toxicity seen in treated animals compared to the control groups indicate that the dosing was adequate for an oncogenicity study.

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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. The study deficiencies did not affect the outcome of the study.

C. STUDY DEFICIENCIES

A NOAEL was not determined in this study. However, a NOAEL of 10 ppm was determined for Fluazinam in mice in an earlier study (see MRID 42208405). The number of female mice reserved for the 78-day satellite study was decreased due to error early in the study, resulting in there being 18/group instead of 20/group. This error had no significant effect on the outcome of the study.

APPENDIX 1

TECHNICAL FLUAZINAM: TOXICITY TO MICE
BY DIETARY ADMINISTRATION FOR 4 WEEKS

MRID 44807211

Dose Selection Study in Mice

MRID NO.: 44807211

Study Type: 4-Week dose-range finding study for 83-2b

Test Material: Technical fluazinam (97.0%, lot no. 1030/91)

Document No: ISK 49/921049

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: Huntingdon Research Centre Ltd, P.O. Box 2, Huntingdon, Cambridgeshire, PE18 6ES, England

Citation: Chambers, P.R., T. Gardner, D. Crook, et. al. (1994) Technical Fluazinam: toxicity to mice by dietary administration for 4 weeks. Contains: final report and addendums 1 through 5.

Report Date: March 7, 1994

Methods/ Results/Conclusion:

Test Animals: Crl:CD-1(ICR)BR mice, 5 - 6 weeks old at study initiation
Source: Charles River Breeding Laboratories (Portage, MI, USA)
Housing: 2/cage

Group Size: 14 males, 14 females

Test Concentrations: 0, 3000, 5000, 7000 ppm in the diet

Test substance intake: Males: 0, 555, 938, 1199; females: 0, 658, 1050, 1404 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 comparable to the control group.

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

Clinical pathology: Hematology parameters including hematocrit, hemoglobin, red cell count, total white cell count, differential white cell count, and platelet count, mean corpuscular hemoglobin concentration, and mean corpuscular volume were measured during the 3rd week of treatment. Slightly higher (27%, $p<0.01$) platelet counts and total white cell counts (20%, $p<0.05$) compared to the control were seen in females at 7000 ppm, but the increased values were still within normal ranges and mainly attributed to low control values. Clinical chemistry investigations during week 4 revealed a slight decrease in serum aspartate aminotransferase activity (32%, $p<0.05$) and an increase in blood glucose (16%, $p<0.5$) compared to the control in males at 7000 ppm. Blood cholesterol levels were slightly decreased in males and increased in females at 5000 and 7000 ppm compared to the controls, but there was no clear dose effect. The clinical chemistry changes were within normal ranges and may not be treatment-related (see Charles River Laboratories Technical Bulletin, Baseline Hematology and Clinical Chemistry Values for Charles River Outbred Mice: Crl:CD-1®(ICR)BR, Summer 1986).

Gross pathology: Treatment-related increased incidences of pale livers and/or accentuated lobular markings on the liver were observed in males at all dose levels and in females at 7000 ppm. Treatment-related slightly enlarged livers were also observed in males and females at 7000 ppm.

Organ weights: The group mean liver weights adjusted for the final body weights were increased by 34, 39, and 50% in males and 31, 34, and 39% in females at 3000, 5000, and 7000 ppm, respectively, compared to the control groups ($p<0.01$). The adjusted group mean kidney weights were also increased in males by 14 and 9% ($p<0.01$) at 5000 and 7000 ppm, respectively, compared to the control, but the increases were not dose-related.

Histopathology: Increased incidences of centrilobular hepatocyte enlargement were seen in all treated mice compared to the controls (males: control, 0/14; all concentrations, 14/14; females: controls, 0/14; 3000 ppm, 9/14; 5000 ppm, 13/14; 7000 ppm, 14/14). The hepatocyte enlargement in males was graded "moderate" for all mice at 7000 ppm and for 1/14 and 2/14 at 3000 and 5000 ppm, respectively; all lesions were graded "minimal" in females. Increased incidences of increased height of kidney cortical tubular epithelium were seen in males at 5000 ppm (2/14) and 7000 ppm (7/14, graded "minimal") compared to the control group (0/14). No treatment-related kidney changes were seen in females.

In an addendum (#5) to this range-finding study (dated 8/27/98), increased incidences and severity of vacuolation of white matter of the brain were seen in males at all dose levels and in females at the high dose. Increased incidences and severity of vacuolation of white matter of the spinal cord were also seen in males at 5000 and 7000 ppm. The distribution and severity of the brain and spinal cord lesions is shown in Table 1.

TABLE 1. Vacuolation of white matter of the brain and spinal cord in mice fed fluazinam for 4 weeks

Tissue	Exposure concentration (ppm)			
	0	3000	5000	7000
Males (n = 14)				
Cerebrum	5(0)[a]	14**(1)	14**(10**)	14**(11**)
Cerebellum/pons/medulla	7(0)	14**(2)	13**(9**)	14***(13**)
Spinal cord	6(0)	10(0)	14**(5*)	14**(8**)
Females (n=14)				
Cerebrum	2(0)	7(0)	5(0)	14**(1)
Cerebellum/pons/medulla	6(0)	7(0)	6(0)	12*(1)
Spinal cord	3	5	1	4(0)

Data taken from Addendum 5, p. 211, MRID 44807211.
[a]Numbers in parentheses are the number of mice with lesion graded "moderate" or "marked, significance calculated by reviewer."
*p<0.05, **p<0.01, Significantly different from control.

Conclusions: The high dietary concentration of 7000 ppm was considered suitable for the high dose in a long-term oncogenicity study. **It is noted that treatment-related vacuolation of the white matter of the brain was clearly observed in male mice at 4 weeks after treatment was initiated at a dietary dose level of 3000 ppm (555 mg/kg/day; lowest dose level tested) and higher. A NOAEL was not demonstrated for this effect (i.e. NOAEL <555 mg/kg/day).**

This study is classified as **Acceptable/Nonguideline** and was used as a dose range-finding study.

APPENDIX 2

PESTICIDE REGULATION (PR) NOTICE 94-5

(Not available electronically)

RAB2300:fluazi15.030

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 (1996)

Reference:

Chambers, P.R. (1996) Technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (vols. 1-13). Contains: final report and addendums 1 & 2. Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671, December 19, 1996. MRID 44807222. Unpublished.

Chambers, P.R. (1998) Addendum report to: Technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (vols. 1-13). Contains: final report and addendums 1 & 2. Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671, August 28, 1998. MRID 44807221. Unpublished.

Gopinath, C. (2000) Pathology Working Group (PWG) report on liver tumours in study no. ISK 50/950671 - technical fluazinam: potential tumorigenic effects in prolonged dietary administration to mice (MRID #s 44807222 & 44007221). Huntingdon Life Sciences, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 50/950671 PWG, August 24, 2000. MRID 45201301. Unpublished.

Chambers, P.R., T. Gardner, D. Crook, et. al. (1994) Technical fluazinam: toxicity to mice by dietary administration for 4 weeks, contains: final report and addendums 1 through 5. Huntingdon Research Centre, LTD., Huntingdon, Cambridgeshire, PE18 6ES, England, Document No. ISK 49/921049, March 7, 1994. MRID 44807211. Unpublished.

A. Experimental Design:

In a carcinogenicity study (MRID 44807222, 44807221), technical grade Fluazinam (97.0% a.i., lot no. 1030/91) was administered to groups of 50 male and 50 female Crl:CD®-1 mice in the diet at concentrations of 0, 1000, 3000 or 7000 ppm. The test diets were given for 97 weeks to females and for 104 weeks to males. These concentrations resulted in mean daily compound intakes of 126, 377 and 964 mg/kg/day for males and 162, 453 and 1185 mg/kg/day for females for 1000 ppm, 3000 ppm and 7000 ppm, respectively. Twenty mice were added to the control and 7000 ppm groups for a satellite study in which the mice were killed and necropsied after treatment for 78 weeks. A Pathology Working Group (PWG) report presenting revised incidences for hepatocellular tumors in the male mice in this study was later submitted (MRID 45201301). A four-week range-finding study in mice was also conducted using 0, 3000, 5000 and 7000 ppm in the diet (MRID 44807211).

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 mice in this study. Female mice in this study, however, showed a statistically significant ($p<0.05$) increased trend for mortality with increasing doses of fluazinam and a statistically significant ($p<0.01$) increased pair-wise mortality at 7000 ppm [6].

The incidences of hepatocellular tumors for the male and female mice in this study shown below in Table 6 were taken from the original study report (MRID 44807222). However, revised incidences of hepatocellular tumors for the male mice in this study were later presented in the PWG report (MRID 45201301) and are shown in Table 7 (Main Group Animals) and in Table 8 (Satellite Group Animals). The CARC considered the data in the PWG report to be the definitive incidence data for the statistical analysis of the hepatocellular tumors in the male mice in this study (see Table 9). It should be noted that this table (Table 9) combined the PWG data from the main group animals (Table 7) and from the satellite group animals (Table 8).

Based on the PWG consensus (Table 9), increased incidences of hepatocellular adenomas, carcinomas and of combined hepatocellular adenomas/carcinomas were observed in the treated male mice in this study. The percentage incidences of hepatocellular adenomas were 17%, 27%, 47% and 30% for the 0, 1000 ppm, 3000 ppm and 7000 ppm male groups, respectively. The increase in the 3000 ppm group was statistically significant ($p<0.01$), whereas the increases at 1000 ppm and 7000 ppm were not statistically significant. The percentage incidences of hepatocellular carcinomas observed in the treated male mice in this study were 2%, 4%, 9% (not statistically significant) and 8% (not statistically significant) for the 0, 1000 ppm, 3000 ppm and 7000 ppm male groups, respectively. The percentage incidences of combined hepatocellular adenomas/carcinomas were 18%, 31%, 49% and 33% for the 0, 1000 ppm, 3000 ppm and 7000 ppm male groups, respectively. The increases in the 3000 ppm group ($p<0.01$) and in the 7000 ppm group ($p<0.05$) were statistically significant, whereas the increase at 1000 ppm was not statistically significant. The male mice in this study showed no statistically significant increasing trends.

Female mice in the 1996 study (see Table 10) had a statistically significant ($p<0.05$) positive trend for combined hepatocellular adenomas/carcinomas. The percentage incidences of combined hepatocellular adenomas/carcinomas were 2%, 0%, 11% and 7% for the 0, 1000 ppm, 3000 ppm and 7000 ppm female groups, respectively. There were no significant differences in the pair-wise comparisons of any of the treated female groups with the controls.

[6] 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. 10-11.

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TABLE 6. Hepatocellular tumors in male and female mice fed Fluazinam for up to 104 weeks [+]
DATA FROM THE ORIGINAL STUDY REPORT (MRID 44807222)

Organ and/or neoplasm	Dietary concentration (ppm)			
	0	1000	3000	7000
Males (n=50)				
Liver/ hepatocellular adenoma	6 (12 %)(a)	13 (26 %)	22**## (44 %)	16**## (32 %)
Liver/ hepatocellular carcinoma	1 (2 %)	2 (4 %)	3 (6 %)	4 (8 %)
Liver/ hepatocellular adenoma and/or carcin.	7 (14 %)	15 (30 %)	23**##(b) (46 %)	18**##(b) (36 %)
Females (n=50)				
Liver/ hepatocellular adenoma	1 (2 %)	0 (0 %)	3 (6 %)	3 (6 %)
Liver/ hepatocellular carcinoma	0 (0 %)	0 (0 %)	1 (2 %)	0 (0 %)
Liver/ hepatocellular adenoma and/or carcin.	1 (2 %)	0 (0 %)	4 (8 %)	3 (6 %)

[+] Uncensored Data.

Data taken from p. 36, pp. 2221-2222 and Table 9, pp. 158-166, MRID 44807222.

(a) Percentage incidence in parentheses

(b) Two mice had both an adenoma and a carcinoma

**$p<0.01$, one-tailed pairwise comparisons against the control group

$p<0.01$, one-tailed trend test using groups with dose 0 up to the dose level for that row

TABLE 7. Hepatocellular tumors in male mice fed Fluazinam for up to 104 weeks +
DATA FROM PATHOLOGY WORKING GROUP REPORT (MRID 45201301)
MAIN GROUP ANIMALS

Organ and/or neoplasm	Dietary concentration (ppm)			
	0	1000	3000	7000
Males				
Liver/ hepatocellular adenoma(ta) **ONLY** $	7 # (14 %)(a)	13 (26 %)	19 ** (38 %)	13 (26 %)
Liver/ hepatocellular carcinoma(ta)	1 (2 %)	2 (4 %)	4 (8 %)	4 (8 %)
Liver/ hepatocellular adenoma(ta) and/or carcinomata(ta)	8 ## (16 %)	15 (30 %)	23** (46 %)	17 * (34 %)
Number of livers examined	50	50	50	50

+ Uncensored Data.

Data taken from p. 9 and pp. 20-25, MRID 45201301

$ Three animals in the 3000 ppm group and two animals in the 7000 ppm group had hepatocellular adenoma(ta) as well as carcinoma(ta)

(a) Percentage incidence in parentheses

* $p<0.05$, one-tailed pairwise comparisons against the control group

** $p<0.01$, one-tailed pairwise comparisons against the control group

# $p<0.05$, one-tailed trend test

$p<0.01$, one-tailed trend test

TABLE 8. Hepatocellular tumors in male mice fed Fluazinam for 78 weeks (Satellite Groups) +
DATA FROM PATHOLOGY WORKING GROUP REPORT (MRID 45201301)
SATELLITE GROUP ANIMALS

Organ and/or neoplasm	Dietary concentration (ppm)			
	0	1000	3000	7000
Males				
Liver/ hepatocellular adenoma(ta) **ONLY**	4	0	0	3
Liver/ hepatocellular carcinoma(ta)	0	0	0	1
Liver/ hepatocellular adenoma(ta) and/or carcinomata(ta)	4	0	0	4
Number of livers examined	20	0	0	20

+ Uncensored Data.

Data taken from p. 10, MRID 45201301

Table 9. Fluazinam - 1996 CD-1 Mouse Study - Males

2000 PWG Consensus – This Table combines the data from the Main Group Animals (from Table 7) and the Satellite Group Animals (from Table 8)

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

	Dose (ppm)			
	0	1000	3000	7000
Adenomas (%)	11[a]/66 (17)	13/48 (27)	22/47 (47)	19/64 (30)
p =	0.078	0.133	0.001**	0.060
Carcinomas (%)	1/66 (2)	2[b]/48 (4)	4/47 (9)	5/64 (8)
p=	0.069	0.382	0.095	0.097
Combined (%)	12/66 (18)	15/48 (31)	23[c]/47 (49)	21[c]/64 (33)
p =	0.069	0.082	0.001**	0.043*

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. 12.

[+] 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 71, dose 0 ppm.
[b]First carcinoma observed at week 79, dose 1000 ppm.
[c]Three animals in each of the 3000 and 7000 ppm dose groups 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 $p < 0.01$.

Table 10. Fluazinam - 1996 CD-1 Mouse Study - Females

Female Hepatocellular Tumor Rates[+] and
Peto's Prevalence Test Results (p values)

	Dose (ppm)			
	0	1000	3000	7000
Adenomas (%)	1/52 (2)	0/33 (0)	3/37 (8)	3[a]/46 (7)
p =	0.053	-	0.128	0.055
Carcinomas (%)	0/29 (0)	0/26 (0)	1[b]/27 (4)	0/13 (0)
p=	0.344	-	0.150	-
Combined (%)	1/52 (2)	0/33 (0)	4/37 (11)	3/46 (7)
p =	0.048*	-	0.068	0.055

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. 13.

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

[a]First adenoma observed at week 76, dose 7000 ppm.
[b]First carcinoma observed at week 97, dose 3000 ppm, in a final sacrifice animal.

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 in 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 44807222) was initiated in 1992 at Huntingdon Research Centre (England), the most directly applicable historical control data would ordinarily be that from the same laboratory for the years 1991-1993. However, the duration of studies during 1991-1993 was 80-96 weeks, which was several weeks less than the 102 weeks in the study under consideration. Therefore, historical control data from the Eye Research Centre (England) for the years 1987-1993, with study durations of 95-104 weeks, were also considered.

For hepatocellular adenomas in the livers of male mice, the range of the percent incidence in the 1991-1993 historical control data (uncensored data) was 8 to 34% and the mean was 15.9% and in the 1987-1993 historical control data (uncensored data) was 0 to 31% and the mean was 11.8% . The percent incidence in the male 7000 ppm group in this study (26% in the uncensored data, Table 7; and 30% in the censored data, Table 9) did not exceed the highest percent incidence in the historical control data for 1991-1993 (34%) or for 1987-1993 (31%). However, the percent incidence in the male 3000 ppm group in this study (38% in the uncensored data, Table 7; and 47% in the censored data, Table 9) did exceed the highest percent incidence in the

historical control data for 1991-1993 (34%) and for 1987-1993 (31%). For hepatocellular carcinomas in the livers of male mice, the range of the percent incidence in the 1991-1993 historical control data (uncensored data) was 2 to 16% and the mean was 8.6% and in the 1987-1993 historical control data (uncensored data) was 4 to 17% and the mean was 9.5%. The percent incidence in the male 7000 ppm group in this study (8% in the uncensored data, Table 7; and 8% in the censored data, Table 9) did not exceed the highest percent incidence in the historical control data for 1991-1993 (16%) or the highest percent incidence in the historical control data for 1987-1993 (17%). The percent incidence in the male 3000 ppm group in this study (8% in the uncensored data, Table 7; and 9% in the censored data, Table 9) also did not exceed the highest percent incidence in the historical control data for 1991-1993 (16%) or the highest percent incidence in the historical control data for 1987-1993 (17%). For combined hepatocellular adenomas/carcinomas in the livers of male mice, the range of the percent incidence in the 1987-1993 historical control data (uncensored data) was 4 to 42% and the mean was 20.8%. The percent incidence in the male 7000 ppm group in this study (34% in the uncensored data, Table 7; and 33% in the censored data, Table 9) did not exceed the highest percent incidence in the historical control data for 1987-1993 (42%), but the percent incidence in the male 3000 ppm group in this study (46% in the uncensored data, Table 7; and 49% in the censored data, Table 9) did exceed the highest percent incidence in the historical control data for 1987-1993 (42%).

C. Non-neoplastic Lesions

Statistically significant ($p<0.01$) increased incidences of several histopathological lesions in the liver of 1000 ppm, 3000 ppm and 7000 ppm male mice were observed in this study. These lesions included altered hepatocytic foci ($p<0.05$ at 1000 ppm), hepatocyte enlargement, pale or vacuolated hepatocyte cytoplasm and aggregates of brown pigmented macrophages. Statistically significant increased incidences of brown pigmented centrilobular hepatocytes and parenchymal inflammatory cells were also observed in the liver of 3000 ppm ($p<0.05$) and 7000 ppm ($p<0.01$) male mice. Statistically significant ($p<0.01$) increased incidences of several histopathological lesions in the liver of 1000 ppm, 3000 ppm and 7000 ppm female mice were also observed in this study. These lesions included hepatocyte enlargement, pale or vacuolated hepatocyte cytoplasm and aggregates of brown pigmented macrophages. A statistically significant increased incidence of altered hepatocyte foci was also observed in the liver of 7000 ppm ($p<0.01$) female mice in this study.

Treatment-related increases in the incidence and severity of vacuolation of the white matter of the brain of male and female mice were observed at 1000 ppm, 3000 ppm and 7000 ppm in this study. Treatment-related increases in the incidence and severity of vacuolation of the white matter of the spinal cord of male and female mice were also observed at 3000 ppm and 7000 ppm. No signs of increased goitrogenic activity were observed in either the male or female mice.

Adequacy of Dosing for Assessment of Carcinogenic Potential

The highest dose level tested in this study (7000 ppm) is equivalent to the limit dose in the 870.4200 guidelines. In addition, the dosing was adequate for a carcinogenicity study based on increased mortality in females, decreased body weight gains in males and females, and liver, brain and spinal cord toxicity in males and females at 7000 ppm. Treatment with fluazinam resulted in a significant increase in mortality in females at 7000 ppm (control, 48%; 7000 ppm, 76%; $p<0.01$). All females were terminated after 97 weeks of treatment because of increased mortality at the high-dose. At 7000 ppm, body weight gain was decreased in males during weeks 4-36 by 32% ($p<0.01$) and food conversion ratios over weeks 9-13 were increased by 86% compared to the controls indicating decreased efficiency of food utilization. At termination, relative liver/body weight ratios were increased in males by 54%, 113% and 182% and in females by 21%, 45% and 109% at 1000, 3000 and 7000 ppm, respectively, compared to the controls ($p<0.01$). Microscopic examination showed increased incidences of histopathological lesions in the liver, brain and spinal cord of males and females (see "Non-neoplastic Lesions", above, for more details).