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PREVENTION, PESTICIDES
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March 29, 2001

MEMORANDUM

PC Code 129098

SUBJECT: Fluazinam - Report of the Cancer Assessment Review Committee

FROM: Sanjivani Diwan
Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Ed Budd, Toxicologist
Registration Action Branch 2
Health Effects Division (7509C)

William Cutchin, Risk Assessor
Science Information Management Branch
Health Effects Division (7509C)

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Fungicide Branch
Registration Division (7505C)

The Cancer Assessment Review Committee met on January 3, 2001 to evaluate the carcinogenic potential of Fluazinam. Attached please find the Final Cancer Assessment Document.

cc: K. Dearfield
R. Hill
Y. Woo
J. Pletcher

014512

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF FLUAZINAM

P.C. Code: 129098

FINAL REPORT

29- March, 2001

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

FLUAZINAM CANCER ASSESSMENT DOCUMENT FINAL REPORT

DATA PRESENTATION: Edwin R. Budd
Edwin R. Budd, Toxicologist

DOCUMENT PREPARATION: Sanjivani B. Diwan
Sanjivani B. Diwan, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke
William Burnam
Marion Copley
Yiannakis Ioannou
Timothy McMahon
Nancy McCarroll
Esther Rinde
Linda Taylor
Yin Tak Woo — See attached sheet

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John M. Pletcher Pathology Consultant — See attached sheet
Lori Brunsman, Statistician

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CONTENTS

EXECUTIVE SUMMARY

On January 3, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluazinam. The studies evaluated included a combined chronic toxicity and carcinogenicity study in Sprague-Dawley rats (1988), a chronic toxicity study in Sprague-Dawley rats (1993), two carcinogenicity studies in CD-1 mice (1988 and 1996), several subchronic toxicity studies in rats and dogs, and a battery of mutagenicity studies. No mode of action studies related to the mechanism of tumor induction in rats or mice were available for review.

In the 1988 rat study, fluazinam was administered in the diet to groups of 60 male and 60 female Sprague-Dawley rats at dietary concentrations of 0, 1, 10, 100 or 1000 ppm (0, 0.04, 0.38, 3.8 or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.9 or 53 mg/kg/day for females, respectively) for up to 104 weeks. In the 1993 rat study, fluazinam was administered to groups of 25 male and 25 female Sprague-Dawley rats at dietary concentrations of 0, 25, 50 or 100 ppm (0, 1.0, 1.9 or 3.9 mg/kg/day for males and 0, 1.2, 2.4 or 4.9 mg/kg/day for females, respectively) for 104 weeks. In the 1988 mouse study, fluazinam was administered to groups of 52 male and 52 female CD-1 mice at dietary concentrations of 0, 0, 1, 10, 100 or 1000 ppm (0, 0, 0.12, 1.12, 10.7 or 107 mg/kg/day for males and 0, 0, 0.11, 1.16, 11.7 or 117 mg/kg/day for females, respectively) for 104 weeks. In the 1996 mouse study, fluazinam was administered in the diet to groups of 50 male and 50 female CD-1 mice at concentrations of 0, 1000, 3000 or 7000 ppm (0, 126, 377 or 964 mg/kg/day for males and 0, 162, 453 or 1185 mg/kg/day for females, respectively) for 97 weeks to females (due to increased mortality) and for 104 weeks to males. A Pathology Working Group (PWG) report presenting revised incidences for hepatocellular tumors in the male mice in this study was later submitted by the Applicant.

The CARC concluded that:

- **There was <u>some evidence</u> that fluazinam was carcinogenic to male rats** because: 1) There were statistically significant positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/ adenocarcinomas. There was also a statistically significant increase by pair-wise comparison of 1000 ppm (40 mg/kg/day) or high dose group with the controls for combined follicular cell adenomas/adenocarcinomas. Because of the significant survival disparities between the control and treated groups of male rats, the tumor data were also analysed by the Peto's Prevalence Test. The results revealed a statistically significant positive trend and a borderline statistically significant (p= 0.056) increase by pair-wise comparison of the 1000 ppm dose group with the controls for thyroid follicular cell adenocarcinomas indicating that there was a malignant component to the thyroid tumors; and 2) The incidences of thyroid gland adenomas at ≥100 ppm (≥3.8 mg/kg/day) and adenocarcinomas at 1000 ppm were slightly outside their respective ranges for the historical controls (range: adenomas, 0%-13%; adenocarcinomas, 0%-5%). However, this increased incidence of thyroid tumors at 100 ppm was not observed in male rats in a more recent 1993 chronic study. The CARC noted that at 1000 ppm, there were increased thyroid weights (at 52 weeks), enlarged thyroids and a slightly increased incidence

of thyroid gland follicular cell hyperplasia at 104 weeks in males. However, the animals in the lower dose groups were not microscopically examined for thyroid lesions unless abnormalities were observed in that organ at gross necropsy. Therefore, the incidences of thyroid tumors in these lower dose groups may have been somewhat misleading (too high). There was no treatment-related increase in tumor incidence in female rats. The highest dose level tested in this study was considered to be adequate and not excessive because there were decreased body weight gains and the histopathological changes observed were not severely adverse. There also was no adverse effect on survival of the treated animals. **The CARC concluded that there was some evidence that the thyroid tumors observed in the male rats were treatment-related.**

- **In the 1988 mouse study, there was clear evidence that fluazinam was carcinogenic to male mice** because: 1) There were statistically significant positive trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas. There were also statistically significant increases by pair-wise comparison of the 1000 ppm (107 mg/kg/day) or high dose group with the controls for hepatocellular adenomas, carcinomas and for combined adenomas/carcinomas; and 2) The incidence of hepatocellular adenomas and hepatocellular carcinomas at 1000 ppm exceeded the range of the historical controls for 1986-1988 (range: 8-23% and 5-13%, respectively). For both males and females at 1000 ppm, the mean liver weights were increased and the histopathological changes were observed in the liver and brain . There were no treatment-related tumors observed in the female mice. The highest dose level tested in this study was considered to be adequate and not excessive because the histopathological changes observed were not severely adverse. There was also no effect on survival or body weight gain of the treated animals. **The Committee concluded that there was clear evidence of treatment-related increases in both benign and malignant liver tumors in the male mice in this study.**

- **In a recent (1996) mouse study, there was equivocal/some evidence that fluazinam was carcinogenic to male mice** because: 1) There were no statistically significant positive trends for hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the male mice. However, there was a statistically significant increase by pair-wise comparison with the controls for hepatocellular adenomas at 3000 ppm (377 mg/kg/day) or mid dose, and combined adenomas/carcinomas at ≥3000 ppm; and 2) The incidence of hepatocellular adenomas at 3000 ppm was outside the historical control range for 1991-1993 (8-34%) and for 1987-1993 (0-31%), but the incidence at 7000 ppm (964 mg/kg/day) was within the range for the comparable historical control data. Similarly, the incidence of combined hepatocellular adenomas/carcinomas at 3000 ppm was outside the historical control range for 1987-1993 (4-42%), but the incidence at 7000 ppm was within the range for the comparable historical control data. The tumorigenic response did not occur in a dose-related manner. The highest dose level tested for the male mice in this study was considered to be adequate and not excessive. The toxicity observed at this dose level (decreased body weight gain of 32% compared to controls and histopathological effects in the liver, brain and spinal cord) was not considered to be life-threatening. The Committee noted that the increase in hepatocellular tumors observed in the male mice in the 1988 mouse study at a dose level of 1000 ppm (107 mg/kg/day) was not confirmed in the 1996 mouse study at the same dose

level (1000 ppm or 126 mg/kg/day) using the same strain of mice. **The Committee concluded that the evidence for carcinogenicity in the liver of male mice in this study was equivocal/some evidence because there was evidence suggesting a possible treatment-related increase in benign liver tumors.** Only a statistically significant positive trend was observed for combined hepatocellular adenomas/carcinomas for the female mice. The highest dose was considered to be excessive for females because there was treatment-related increase in mortality. **The Committee also determined that the hepatocellular tumors observed in female mice at 7000 ppm (1185 mg/kg/day) occurred at an excessively toxic dose which may have resulted in indirect effects that may not have been present at lower doses.**

- A battery of acceptable mutagenicity assays indicated that fluazinam was not mutagenic .

- No mode of action studies were available that were related to the mechanism of tumor induction in rats or mice. There are insufficient data to determine whether the thyroid gland follicular cell tumors observed in rats may be due to a disruption in thyroid-pituitary homeostasis.

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified fluazinam into the category **"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on the following weight-of-the-evidence considerations:

1. There was some evidence in that fluazinam induced an increase in thyroid gland follicular cell tumors in male rats, but not in female rats. In one study in mice, there was clear evidence that an increased incidence of hepatocellular tumors observed in the male mice was treatment-related. In another study in mice, there was equivocal/some evidence that fluazinam may have induced an increase in hepatocellular tumors in the male mice. Increases in hepatocellular tumors observed in the female mice in the latter study were not statistically significant and some occurred at an excessively toxic dose level. The thyroid gland follicular cell tumors of concern were seen only in male rats and the hepatocellular tumors of concern were seen only in male mice.

2. Fluazinam was negative in mutagenicity assays.

The Committee determined that the quantification of human cancer risk is not required.

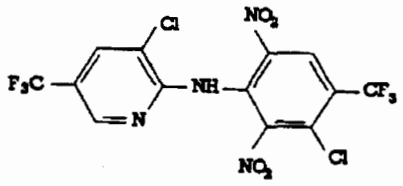
I. INTRODUCTION

On January 3, 2001, the Cancer Assessment Review Committee (CARC) of the Health Effects Division (HED) of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluazinam. Edwin Budd of the Registration Action Branch 2 presented the relevant data and information pertaining to the evaluation of the carcinogenic potential of this chemical.

II. BACKGROUND INFORMATION

Fluazinam (P.C. Code: 129098, CAS No.: 79622-59-6, 3-chloro-N-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine), also known as B-1216, IKF-1216 or PP192, is a new active ingredient proposed for use as a fungicide on peanuts, potatoes and wine grapes at this time. No residential uses have been requested. It is presumed, however, that additional food and/or non-food uses may be proposed in the future.

Figure 1. Chemical Structure of Fluazinam



III. EVALUATION OF CARCINOGENICITY EVIDENCE

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

Reference:

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

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

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

A. Experimental Design

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

B. Discussion of Tumor Data

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of fluazinam in the male or female rats (Brunsman, 2000). Incidences of the thyroid gland follicular cell neoplasms seen in the male rats are presented in Table 1. The thyroid gland neoplasms described in the study report as "follicular cystadenomas" are included in the table under the category of "follicular adenomas". The incidence of thyroid gland follicular cell adenomas in the male rats was 8%, 9%, 13%, 15% and 17% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. In none of the treated groups was the increased incidence statistically significant by trend or pair-wise comparison analyses with the control group. The incidence of thyroid gland follicular cell adenocarcinomas was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. There was a statistically significant ($p<0.05$) positive trend. The increased incidence at $\geq$100 ppm was not statistically significant by pair-wise comparison with the control group. The incidence of thyroid gland follicular cell combined adenomas/adenocarcinomas was 8%, 9%, 13%, 18% and 23% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. Male rats had a statistically significant increasing ($p<0.05$) trend and a significant increase ($p<0.05$) by pair-wise comparison of the 1000 ppm group with the controls, for thyroid gland follicular cell combined adenomas/adenocarcinomas.

Historical Control Data for Thyroid Gland Follicular Cell Neoplasms in Male Sprague-Dawley Rats: Uncensored Data from 20 studies performed during 1981 and 1990 (study durations: 101-111 weeks) at Huntingdon Life Sciences, Huntingdon, England, from MRID 45150201

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

Based on the comparison with the above historical control data for male rats, the incidence of thyroid gland follicular cell adenomas at 100 ppm (15%) and at 1000 ppm (17%) exceeded the highest percent incidence in the historical control data (13%). For thyroid gland follicular cell adenocarcinomas in male rats, the incidence at 1000 ppm (6%) exceeded the highest percent incidence in the historical control data (5%).

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

Male Thyroid Gland Follicular Cell Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values)- (Brunsman, 2000)

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

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

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

[a]First adenoma observed at week 70, dose 100 ppm.

[b]First carcinoma observed at week 68, dose 1000 ppm.

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

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

The CARC noted that although there were no statistically significant survival disparities between the control and treated groups of male rats in this study (Cox or Generalized K/W Test), nevertheless, the mortality of the control group was 15% higher than that of the males at 1000 ppm. Therefore, in addition to the Exact Trend Test and Fisher's Exact Test, the CARC also analysed the incidences of thyroid gland follicular cell neoplasms in male rats by Peto's Prevalence Test (Brunsman, 2001; Table 2).

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

Male Thyroid Gland Follicular Cell Tumor Rates[+]
and Peto's Prevalence Test Results (p values)- (Brunsman, 2001)

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

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

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

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

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

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

The results of the Peto's Prevalence Test showed a statistically significant positive trend ($p<0.05$) and a borderline statistically significant ($p= 0.056$) increase by pair-wise comparison of the 1000 ppm male group with the controls (7% vs 0% in controls) for follicular cell adenocarcinomas in males, indicating the increased incidence of thyroid tumors had a malignant

component. There was also a statistically significant ($p<0.05$) increase by pair-wise comparison of the high dose male group with the controls (26% vs 9% in controls) for combined follicular cell adenomas/adenocarcinomas. The CARC determined that there was some evidence that the increase in thyroid tumors was treatment related.

In addition to thyroid tumors, increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats (Refer to Budd, 2000). The incidence of pancreatic islet cell adenomas in the male rats in this study was 8%, 10%, 24%, 14% and 18% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. In none of the treated groups was the increased incidence statistically significant by trend or pair-wise comparison analyses with the control group. In addition, the observed incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (range: 0 % to 28%; mean: 13.8%; data from 20 comparable studies at the same testing laboratory, MRID 45150201). This type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Thus, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats was not considered to be treatment related. Further, no pancreatic islet cell adenocarcinomas were observed in any of the treated male rats in this study. High incidences of pituitary gland adenomas in treated males (48% to 71%; controls, 60%) and in treated females (52% to 86%; controls. 68%) and of mammary gland fibroadenomas in treated females (79% to 94%; controls 87%) were also observed in all groups, including controls. These neoplasms were not considered to be treatment related. A noteworthy increase of tumors was not observed in any other tissues in the treated male or female rats in this study.

C. Non-neoplastic Lesions

Histopathological examination revealed a possible increase in goitrogenic activity in rats. At 1000 ppm, absolute and relative thyroid weights were increased ($p<0.01$) in males at 52 weeks (but not at 104 weeks). Absolute mean thyroid weights at 52 weeks were 29.6, 31.7, 27.2, 31.0 and 42.4 g for the 0, 1, 10, 100 and 1000 ppm dose groups respectively. Relative mean thyroid weights were 3.91 and 6.40 g for the 0 and 1000 ppm dose groups, respectively. Absolute liver weights were increased by at least 7-27% and relative weights by 17-63% in both sexes at 1000 ppm. An increased incidence of enlarged thyroids ($p<0.05$) was also observed during gross examination of the males at 104 weeks. The incidences were 2/50, 2/50, 4/50, 4/50 and 9/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively. In addition, a slightly increased incidence (not statistically significant) of thyroid follicular cell hyperplasia was observed at 104 weeks in males (1/50, 2/35, 1/40, 0/35 and 4/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively). A slightly increased incidence (not statistically significant) of thyroid follicular cell hyperplasia was also observed at 104 weeks in females (1/50, 0/25, 0/27, 0/32 and 5/50 for the 0, 1, 10, 100 and 1000 ppm dose groups, respectively).

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

of the pancreas was observed (controls, 3/50; 100 ppm, 13/50). Centrilobular hepatocyte vacuolation and centrilobular fat were also seen in 1000 ppm group male and female rats at interim sacrifice.

At the highest dose tested in this study (1000 ppm), gross lesions in the liver consisted of pale, swollen or accentuated markings on the livers in males and enlarged, pitted, or mottled livers in females. The statistically significant ($p<0.01$) increased incidences of several histopathological liver lesions in both male and female rats were observed. These lesions included centrilobular sinusoidal dilatation, pericholangitis, eosinophilic hepatocytes, centrilobular hepatocyte rarefaction and vacuolation, and centrilobular fat. Statistically significant ($p<0.05$) increased incidences of bile duct hyperplasia were also observed in both males and females and centrilobular hepatocyte necrosis in females. In addition, statistically significant increases in males were observed in the following histopathological lesions: pneumonitis and alveolar epithelialization in lungs, exocrine atrophy in pancreas, cortical tubular basophilia in kidney, and atrophy of testes. In females, statistically significant increases were observed in the following histopathological lesions: alveolar epithelialization in lungs; and exocrine atrophy, acinar epithelial vacuolation and acinar epithelial fat in the pancreas.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CARC considered the dosing at the highest dose level to be adequate but not excessive based on decreased body weight gain in males at 1000 ppm and the histopathological lesions observed at ≥100 ppm dose levels. These effects were not considered to be severely adverse.

At 1000 ppm, males weighed 6–16% ($p<0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed ≤8% less food than controls at each weekly interval. Females weighed 7–24% ($p<0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed ≤18% less food than controls at each weekly interval. In addition, treatment-related histopathological changes were noted in the following organs: liver, pancreas, lungs, and possibly thyroid gland in both sexes, and kidneys and testes in males. The primary target organ appeared to be the liver. Liver toxicity was manifested by increased organ weight at 1000 ppm and increased incidences of gross and microscopic lesions at ≥100 ppm dose levels. Histopathological findings observed at 1000 ppm in the liver, pancreas, lungs and thyroid gland in males and females, and in the kidney and testes of males are described under "Non-neoplastic Lesions".

2. Chronic Toxicity Study in Sprague-Dawley Rats

Reference:

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

A. Experimental Design

In a chronic oral toxicity study (MRID 44839901), technical grade fluazinam (95.3% a.i., Batch # 8412-20) was administered to 25 Crl:CD®(SD)BR rats/sex/dose in the diet at dose levels of 0, 25, 50 or 100 ppm for 104 weeks (0, 1.0, 1.9 or 3.9 mg/kg/day for males; 0, 1.2, 2.4 or 4.9 mg/kg/day for females, respectively).

B. Discussion of Tumor Data

Selected neoplastic microscopic findings in male and female rats are presented in Table 3. No increase in the incidence of thyroid gland adenomas or adenocarcinomas was observed in the treated male or female rats in this study. In addition, no other neoplastic lesions were observed in the treated male or female rats in that could be related to the test material. For female rats, an increased incidence of pituitary gland adenocarcinomas (5/25 vs 1/24 for controls) at 1000 ppm, mammary gland fibroadenomas (15/20 vs 8/15 for controls) and adenocarcinomas (7/20 vs 2/15 for controls) at 100 ppm were also of no concern to the Committee because toxicologically significant increases in these same types of tumors were not observed in the combined chronic toxicity/carcinogenicity study in rats at doses up to 1000 ppm. (MRID 42248620). Additional findings (Table 3) for females were for the pancreas. However, slight increases in these same tumor types were observed in male rats and not female rats in the combined chronic toxicity/carcinogenicity study in rats (MRID No. 42248620).

C. Non-neoplastic Lesions

No clinical signs of toxicity were observed, and survival rates were unaffected by treatment.

Among males (decedent [those not surviving to study termination]+ terminal) at 100 ppm, the overall severity of testicular tubular atrophy was slightly increased in the high-dose males (severity grade of 3.1) as compared with the controls (severity grade of 2.6). Because testicular atrophy was also an effect noted in male rats following dietary treatment with 100 or 1000 ppm in the previously conducted chronic toxicity/carcinogenicity study (MRID 42248620), it was considered an effect of treatment in this study.

At the highest dose level tested (100 ppm), the only signs of possibly increased goitrogenic activity were slightly increased incidences of thyroid gland follicular cell hyperplasia at 104 weeks in males (0/24, 0/12, 0/18 and 2/25, not significant) and in females (0/25, 0/13, 0/7 and 2/25, not significant) at 0, 25, 50 and 100 ppm, respectively.

Table 3 Selected neoplastic microscopic findings in male and female rats fed B1216 for up to 104 weeks

Organ/lesions	Dietary concentration (ppm)			
	0	25	50	100
Males				
Pituitary gland, No. animals examined	25	18	20	25
Pituitary adenoma	10	10	7	9
Pituitary adenocarcinoma	0	0	0	1
Combined	10	10	7	10
Pancreas, No. animals examined	25	17	20	25
Islet cell adenoma	4	7	2	5
Islet cell carcinoma	2	0	0	0
Combined	6	7	2	5
Thyroid gland, No. animals examined	24	12	18	25
Follicular adenoma	3	1	0	2
Follicular carcinoma	0	1	0	1
Combined	3	2	0	3
Mammary gland, No. animals examined	4	3	1	2
Fibroadenoma	1	0	0	0
Adenocarcinoma	0	1	0	0
Females				
Pituitary gland, No. animals examined	24	21	18	25
Pituitary adenoma	11	16	16	11
Pituitary adenocarcinoma	1	1	0	5
Combined	12	17	16	16
Pancreas, No. animals examined	25	25	25	25
Islet cell adenoma	0	1	1	1
Islet cell carcinoma	0	0	1	1
Combined	0	1	2	2
Thyroid gland, No. animals examined	25	13	7	25
Follicular adenoma	0	0	0	0
Follicular carcinoma	0	0	0	1
Combined	0	0	0	1
Mammary gland, No. animals examined	15	20	19	20
Fibroadenoma	8	6	6	12
Fibroadenoma with epithelial atypia	0	1	4	3
Combined	8	7	10	15
Adenocarcinoma	2	3	4	7

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

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

No treatment-related effects on mean absolute body weights, body weight gain, food consumption, or water consumption were noted. No treatment-related differences were observed in hematology or urinalysis, and treatment-related clinical chemistry changes were limited to a transient increase in total serum cholesterol in high-dose females at week 52 (154% of controls, $p<0.01$). Relative liver weights were increased in high dose females at study termination (124% of controls; $p<0.01$), but were not accompanied by any histopathological correlates. The increased relative liver weights and transient increase in cholesterol were, therefore, not considered adverse. **The dose levels used in this study were not adequate for assessment of carcinogenic potential.** This study, however, was not intended to be a carcinogenicity study. The dose levels used in this study were selected to determine the NOAEL for non-neoplastic effects in Sprague-Dawley rats following chronic oral exposure to fluazinam.

3. Carcinogenicity Study in CD®-1 Mice (1988)

Reference:

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

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

A. Experimental Design

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

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

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of fluazinam in the male or female mice (Brunsman, 2000).

A summary of the incidences of hepatocellular neoplasms seen in the male mice is given in Table 4. There were significant positive trends ($p<0.05$ or $p<0.01$) and significant ($p<0.05$ or $p<0.01$) increases by pair-wise comparisons of the 1000 ppm group with the combined control groups for hepatocellular adenomas (34% vs 16% in combined controls), hepatocellular carcinomas (34% vs 19% in combined controls), and combined hepatocellular adenomas and/or carcinomas (62% vs 33% in combined controls). The CARC considered the increased incidence of liver tumors in male mice to be treatment-related. There were no compound-related hepatocellular tumors in the treated female mice in this study.

Table 4. Fluazinam - 1988 CD-1 Mouse Study - Males
Male Hepatocellular Tumor Rates[+] and Exact Trend
Test and Fisher's Exact Test Results (p values)- (Brunsman, 2000)

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

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 56, dose 1000 ppm.
[b]First carcinoma observed at week 56, dose 1000 ppm.
[c]Two animals in each of the control, 1 and 100 ppm dose groups had both an adenoma and a carcinoma.
[d]One animal in the 10 ppm dose group had both an adenoma and a carcinoma.
[e]Three animals in the 1000 ppm dose group had both an adenoma and a carcinoma.
Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Historical control data for hepatocellular tumors in male CD-1 mice are presented 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 (MRID 42208405) was initiated in 1985 at Huntingdon Research Centre (England), the most directly applicable historical control data are those from the same laboratory for the years 1981-1983 and 1986-1988 (first 2 columns in Table 5). For males at 1000 ppm, the percent incidence for hepatocellular adenomas (34%) exceeded the highest percent incidence in the historical control data for 1981-1983 (27%) and for 1986-1988 (23%). For hepatocellular carcinomas the percent incidence (34%) did not exceed the highest percent incidence in the historical control data for 1981-1983 (38%), but did exceed the highest percent incidence in the historical control data for 1986-1988 (13%).

The incidence of lung adenoma in female mice at 1000 ppm (8%) was comparable with the incidence in the combined control groups (7%; refer to Budd, 2000) and was within the range for the historical controls (2%-21%; mean percent incidence: 6.4%; MRID 42208405). There was a statistically significant ($p = 0.047$) positive trend for lung adenocarcinoma , but the incidence at 1000 ppm was not significantly different by pair-wise comparison with the combined control groups (15% vs 10% in the combined controls). Moreover, the incidence was within the range for the historical controls (2%-15%; mean percent incidence: 11.2% based on nine studies performed at Huntingdon Research Centre, Huntingdon, England during 1981 and 1983 with

study duration of 97-108 weeks, MRID 42208405). Except for histiocytic sarcomas, the incidence of no single type of uterine tumor was increased in treated mice at any dose level when compared to at least one of the control groups. For histiocytic sarcoma, a slight increase was observed at 1000 ppm (10% vs 2% in combined controls). The CARC did not consider either the lung adenocarcinomas or the uterine histiocytic sarcomas observed in the treated female mice to be treatment-related. (Refer to Budd, 2000 for details regarding the incidences of lung and uterine tumors in female mice).

C. Non-neoplastic Lesions

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

A statistically significant increased incidence of cystic follicles of the thyroid gland was observed at 100 ppm in male mice (52% vs 23% in combined controls, $p<0.01$) and in female mice (33% vs 16% in combined controls, $p<0.05$). The CARC determined that these thyroid gland changes in male and female mice were not treatment-related and did not support the finding of thyroid gland follicular cell tumors in the rat study on fluazinam (MRID 42248620).

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

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CARC considered the dosing in this study to be adequate and not excessive based on toxicity not considered to be severely adverse and there was no effect on survival of treated animals. There was liver and brain toxicity observed in the male and female mice at 1000 ppm. The group mean liver weights (adjusted for body weight) were statistically significantly ($p<0.01$) increased in males and females by 45% and 30%, respectively, at 1000 ppm and also in females by 15% at 100 ppm ($p<0.01$). Microscopic examination of livers also demonstrated several treatment-related lesions in males and females that are described under "Non-neoplastic Lesions". Treatment-related increases in the incidences (in males) and severity (in both sexes) of vacuolation of white matter of the brain was observed at 1000 ppm compared to the control groups.

4. 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.

A. Experimental Design:

In a more recent 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 at dietary 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).

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 mortality at 7000 ppm by pair-wise comparison with the controls (Brunsman, 2000).

The incidences of hepatocellular tumors for the male and female mice (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 Pathology Working Group (PWG) report (MRID 45201301) and are shown in Table 7 (Main Group Animals) and in Table 8 (Satellite Group Animals).

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

The CARC considered the findings 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. Therefore, the tumor incidences presented in Table 9 represent the combined 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 combined hepatocellular adenomas/carcinomas were observed in the treated male mice. 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 ($p<0.01$ by pair-wise comparison with the controls), 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 male mice showed no statistically significant increasing trends for combined hepatocellular adenomas/carcinomas; however, increases in the 3000 ppm group ($p<0.01$) and in the 7000 ppm group ($p<0.05$) were statistically significant by pair-wise comparison with the controls.

Table 9. Fluazinam - 1996 CD-1 Mouse Study - Males (2000 PWG Consensus)
Male Hepatocellular Tumor Rates[+] and Exact Trend
Test and Fisher's Exact Test Results (p values)- (Brunsman, 2000)

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

[+] 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 $p < 0.01$.

Historical control data for hepatocellular adenomas and carcinomas in male CD-1 mice have been presented previously in Table 5.

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 those 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 male mice, the percent incidence hepatocellular adenomas at 7000 ppm (30%, Table 9) did not exceed the range for the historical controls, however, the percent incidence at 3000 ppm (47%, Table 9) did exceed the highest percent incidence in the historical control data for 1991-1993 for 1991-1993 (range: 8%-34%; mean: 15.9%) or for 1987-1993 (range: 0%-31%; mean 11.8%). For hepatocellular carcinomas, the percent incidence at 3000 (9%) and 7000 ppm (8% , Table 9) did not exceed the range for the historical control data for 1991-1993 (2%-16%; mean: 8.6%) or for 1987-1993 (4%-17%; mean: 9.5%). For combined hepatocellular adenomas/carcinomas, the percent incidence at 7000 (33%, Table 9) did not exceed the range for the historical control data for 1987-1993 (4%-42%; mean: 20.8%), but the percent incidence at 3000 ppm (49%, Table 9) did exceed the highest percent incidence in the historical control data for 1987-1993 (42%).

Female mice (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 was a borderline statistically significant increasing trend for hepatocellular adenomas ($p=0.053$) and increases by pair-wise comparisons of the 7000 ppm dose group with the controls for hepatocellular adenomas and combined adenomas/carcinomas ($p=0.055$ for both).

C. Non-neoplastic Lesions

Statistically significant ($p<0.01$) increased incidences of several histopathological liver lesions were observed in male mice at ≥1000 ppm. 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 liver lesions were also observed in female mice at ≥1000 ppm. 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 observed at 7000 ppm ($p<0.01$).

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 and in the white matter of the spinal cord of male and female mice at ≥3000 ppm . No signs of increased goitrogenic activity were observed in either the male or female mice.

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

Female Hepatocellular Tumor Rates[+] and
Peto's Prevalence Test Results (p values)- (Brunsman, 2000)

	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

[+] 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 $p < 0.01$.

D. Adequacy of Dosing for Assessment of Carcinogenic Potential

The CARC considered the dosing at the highest dose level (7000 ppm) to be adequate in males based on decreased body weight gains, and liver, brain and spinal cord toxicity at 7000 ppm which were not considered to be severely adverse. This dose is equivalent to the limit dose in the 870.4200 guidelines. The highest dose was considered to be excessive in females because of a significant increase in mortality (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 efficiency was decreased over weeks 9-13. 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).

IV. TOXICOLOGY DATA

5. Metabolism

In a metabolism study (MRID 44807233), fluazinam (IKF-1216) was administered by gavage at single doses of 0.5 mg/kg or 50 mg/kg, or for 14 days at repeated doses of 0.5 mg/kg/day. In addition to nonlabeled IKF-1216 (lot no. T9002, 99.6% purity), [^{14}C]-IKF-1216 labeled on the phenyl moiety (lot no. 93-5, 98% purity, sp. act. 57.3 mCi/mmol) or on the pyridyl moiety (lot no. 93-90, 98% purity, sp. act. 66.2 mCi/mmol) was also administered in some studies to assess metabolic cleavage of the phenyl or pyridyl ring of the test material. Experimental groups were established for overall distribution/excretion assessment and for analysis of biliary secretion. The metabolic profiles of urine, feces and bile were examined and major metabolites were identified.

There were no treatment-related deaths in the rats. Overall recovery of the administered radioactivity (reported in MRID Nos. 43521006, 43521007, 43521008) was acceptable (93-104%). Only 33-40% of the orally administered dose was absorbed. Unabsorbed parent (60-67% of the orally administered dose) was excreted in the feces. Excretion via the urine was minor. DAPA (4-chloro-N^2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]-5-(trifluoromethyl)-1,2,3-benzenetriamine) glucuronide and AMPA (4-chloro-N^2-[3-chloro-5-(trifluoromethyl)-2-pyridinyl]-3-nitro-5-(trifluoromethyl)-1,2-benzenediamine) mercapturate, the major urinary metabolites, represented only 0.05-0.39% of the administered dose. Radioactivity in the feces represented most of the administered dose (89-100%). A considerable enterohepatic circulation was observed. Identified fecal metabolites, however, represented only 11-69% of the administered dose. For all dose groups, most of the fecal radioactivity appeared to reside with unextractable components in the post-extraction solids (PES). Further analysis of the PES components using base hydrolysis indicated that most of this radioactivity could be attributed to hydrolysis products of AMPA and DAPA. PES radioactivity was also greatest for the low-dose group which was consistent with the lower overall accounting of identified metabolites for this group.

Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the administered dose. With the exception of the low-dose group, parent compound represented most of the identified radioactivity in the feces. AMPA and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the administered dose (except for high-dose female rats where AMPA accounted for 10%). There was minimal retention of radioactivity in the tissues.

AMPA mercapturate and DAPA glucuronide were the major biliary metabolites but represented <4% of the administered dose. Total biliary radioactivity, however, represented 25-34% of the administered dose. Analysis of chromatograms indicated that numerous other metabolites were present in the bile but were individually of insufficient quantity to allow for characterization.

Metabolite profiles from administration of different label positions (pyridyl and phenyl) indicated that there was no metabolic cleavage of the ring structures. Minor quantitative differences in metabolite recovery were observed between genders but were not of sufficient magnitude to suggest biologically relevant differences in the metabolism of fluazinam.

6. Mutagenicity

There are six acceptable mutagenicity studies on technical grade fluazinam. Together, they satisfy the revised mutagenicity guidelines of 1991 (OPP Pesticide Assessment Guidelines, Subdivision F, Series 84, Addendum 9) which are applicable to all new active ingredients. Results in all six studies were negative for mutagenic potential.

Salmonella typhimurium Reverse Mutation Assay

1) In a reverse gene mutation assay in bacteria, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(*uvr*A) of *E. coli* were exposed to fluazinam technical (Lot No. 8412-20, 95.3% a.i.) in dimethyl sulfoxide (DMSO). In the absence of mammalian metabolic activation (S9-mix), the *Salmonella* strains were exposed to fluazinam concentrations up to 2.0 ug/plate and *E. coli* WP2(*uvr*A) was exposed to concentrations up to 250 ug/plate. In the presence of S9-mix, the *Salmonella* strains were exposed to fluazinam concentrations up to 100 ug/plate and *E. coli* WP2(*uvr*A) was exposed to concentrations up to 500 ug/plate. There was no evidence of induced mutant colonies over background without or with metabolic activation. Fluazinam was negative up to cytotoxic concentrations for mutagenic potential in this study (MRID 42270605).

2) In a second reverse gene mutation assay in bacteria utilizing a different lot of the test material, strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(*uvr*A) of *E. coli* were exposed to fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO. In the absence of S9-mix, the *Salmonella* strains were exposed to fluazinam concentrations up to 1.0 ug/plate and *E. coli* WP2(*uvr*A) was exposed to concentrations up to 250 ug/plate. In the presence of S9-mix, the *Salmonella* strains were exposed to fluazinam concentrations up to 100 ug/plate and *E. coli* WP2(*uvr*A) was exposed to concentrations up to 500 ug/plate. There was no evidence of induced mutant colonies over background without or with metabolic activation. Fluazinam was

negative up to cytotoxic concentrations for mutagenic potential in this study (MRID 42270604).

Mammalian Cells in Culture Forward Gene Mutation Assay

3) In a L5178Y/TK +/- mouse lymphoma forward gene mutation assay using a micro-suspension protocol, mouse lymphoma cell cultures were exposed to fluazinam technical (Lot No. A629, 98.4% a.i.) in DMSO, at concentrations up to 4 ug/ml (test 1) and up to 0.3 ug/mg (test 2) in the absence of S9-mix and at concentrations up to 12.5 ug/ml (test 1) and up to 9 ug/ml (test 2) in the presence of S9-mix. Cells were harvested at 3 hours (test 1) and 24 hours (test 2) after the start of treatment in the nonactivated studies and at 3 hours (tests 1 and 2) after start of treatment in the S9-activated studies. No appreciable increases in the mutant frequency were observed in any of the tests, either in the absence or the presence of S9-mix. Fluazinam was negative up to cytotoxic doses for mutagenic potential in this study (MRID 45261801).

4) In a second L5178Y/TK+ mouse lymphoma forward gene mutation assay using a micro-suspension protocol, mouse lymphoma cell cultures were to fluazinam technical (Batch No. KGL 3147/5, ES 4390; ≥ 95% a.i.) in DMSO, at concentrations up to 3 ug/ml (test 1) and up to 5 ug/mg (test 2) in the absence of S9-mix and at concentrations up to 5 ug/ml in the presence of S9-mix. Cells were exposed to the test compound, negative/solvent or positive controls for 4 hours (nonactivated) or for 4 hours (S9-activated). There was no evidence of induced mutant colonies over background in any of the tests, either in the absence or the presence of S9-mix. Fluazinam was negative up to cytotoxic doses for mutagenic potential in this assay (MRID 45156902).

5) In an *in vitro* mammalian cell cytogenetics assay, Chinese hamster lung (CHL) fibroblasts cultures were exposed to fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO, at concentrations of 1, 2 and 4 ug/ml in the absence of S9-mix and at concentrations of 2.38, 4.75 and 9.5 ug/ml in the presence of S9-mix. Cells were harvested 24 and 48 hours after start of treatment in nonactivated studies and at 24 hours after start of treatment in the S9-activated studies. There was no evidence of chromosomal aberrations induced over background without or with metabolic activation. Fluazinam was negative for mutagenic potential in this study (MRID 42270606).

In vivo Cytogenetics

6) In a mouse bone marrow micronucleus assay, five mice/sex/dose were treated once via oral gavage with fluazinam technical (Lot No. 8412-20, 95.6% a.i.) in olive oil at a dose of 2000 mg/kg in an initial micronucleus test and at doses of 500, 1000 and 2000 mg/kg in a second micronucleus test. Bone marrow cells were harvested at 24, 48 and 72 hours post-treatment in the first test and at 24 hours post-treatment in the second test. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any fluazinam concentration or treatment time used in this study. Fluazinam was negative for clastogenic or aneugenic potential up to the limit dose in this assay (MRID 44807224).

In addition, there are two mutagenicity studies on technical grade fluazinam that are unacceptable for regulatory purposes, but do support the negative conclusions from the acceptable assays.

1) An autoradiographic DNA repair test on rat hepatocytes was classified unacceptable because of several serious deficiencies in the conduct of the study. The study was apparently negative for mutagenic potential (MRID 45156901).

2) In a differential killing/growth inhibition assay in bacteria, strains H17 (rec+) and M45 (rec-) of *Bacillus subtilis* were exposed to fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO on paper disks at concentrations up to 0.3 ug/disk in the absence of S9-mix and at concentrations of up to 30 ug/disk in the presence of S9-mix. The S9-fraction was obtained from phenobarbital + 5,6-benzoflavone induced male Sprague-Dawley rat liver. The study was classified unacceptable because one replicate plate per dose were used. The study was apparently negative for mutagenic potential up to cytotoxic doses (MRID 42270607).

7. Structure Activity Relationships

Fluazinam apparently has a unique chemical structure. HED is not currently aware of any structural analogs for this chemical.

8. Subchronic and Chronic Toxicity

In subchronic and chronic toxicity studies in rats, dogs and mice, the liver appeared to be the primary target organ. The summaries for selected studies are presented below:

A. Subchronic toxicity

In a 90-day Acceptable/guideline subchronic oral toxicity study (MRID 42248610, 44807214), technical grade fluazinam (98.5% a.i.) was administered in the diet to 10 CD rats/sex/dose level at dose levels of 0, 2, 10, 50, or 500 ppm for 13 weeks (0, 0.15, 0.77, 3.8, or 38 mg/kg/day for males; 0, 0.17, 0.86, 4.3, or 44 mg/kg/day for females).

Treatment-related effects noted at 500 ppm in comparison to controls included statistically significant increases in organ weights as follows: liver (males : absolute weights: 8 % (not significant) and relative liver/body weight: 11%), lungs (females: absolute weights:18 % and relative lung/body weight: 25%), and uterus (females: absolute weights: 36 % and relative uterus/body weight: 43%). Statistically significant compound-related histopathological lesions were observed in the livers of 500 ppm males (increased incidences of periacinar hepatocyte hypertrophy and sinusoidal chronic inflammation).

The NOAEL in this study is 50 ppm (3.8 mg/kg/day in males and 4.3 mg/kg/day in females). The LOAEL is 500 ppm (38 mg/kg/day in males and 44 mg/kg/day in females), based on increases in absolute and relative liver weights in males and increases in histopathology in the liver of males

(increased incidences of periacinar hepatocyte hypertrophy and sinusoidal chronic inflammation).

In another Acceptable-guideline subchronic oral toxicity study (MRID 42248611), 4 beagle dogs/sex/dose received fluazinam (98.5% a.i.) daily via gelatin capsule at doses of 0, 1, 10 or 100 mg/kg/day for 90 days.

At 100 mg/kg/day, retinal effects of slight hyper-reflection and slight-to-moderate grey mottling of the tapetal fundus in all males and females during at least two of the three ophthalmologic examinations (7, 10 and/or 13 weeks), increased serum plasma alkaline phosphatase levels (~2-fold, largely due to 1 female), increased SGPT in 1 female (~2 to 3-fold), increased absolute/relative liver weights (males 31%/34% and females 33%/36% above controls), hepatic coagulative necrosis (1 male, focal; 2 females, multifocal, all slight, vs. 0, controls) and slight to moderate bile duct hyperplasia with/without cholangiofibrosis (2 males and 2 females) were observed. The LOAEL is 100 mg/kg/day, based on retinal effects, possible increased serum alkaline phosphatase in females, increased relative liver weight and liver histopathology and possible marginal increase in vacuolation of the cerebral white matter. The NOAEL is 10 mg/kg/day.

B. Chronic toxicity

In a combined chronic toxicity/carcinogenicity (MRID 42248620, 44807223, 45150201), no treatment-related effects were observed in rats receiving the 1- or 10-ppm diets (Refer to page 2 for details regarding the dose levels used). Treatment related microscopic findings were seen in the following organs: liver, pancreas, lungs, and possibly thyroid gland in both sexes, kidneys and testes in males, and lymph nodes in females, but the primary target appeared to be the liver (Refer to pages 4 and 5 for details). The lowest-observed-adverse-effect level (LOAEL) for B-1216 was 100 ppm (3.8 mg/kg/day for males and 4.9 mg/kg/day for females) based on liver toxicity in both sexes, testicular atrophy in males and pancreatic exocrine atrophy in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm (0.38 mg/kg/day for males and 0.47 mg/kg/day for females).

In a chronic feeding study in rats (MRID 44839901; 44807213; refer to page 7 for details regarding the dose levels used), there was increase in relative liver weights at study termination in females at 100 ppm (124% of controls; $p<0.01$), but were not accompanied by any histopathological correlates. Treatment with fluazinam appeared to affect the testes. In microscopic examination of all 100 ppm males (decedent + terminal), overall severity of testicular tubular atrophy was increased (3.1) as compared with controls (2.6). A LOAEL of 100 ppm (3.9 mg/kg/day) was identified for male rats based on increased testicular atrophy, with a corresponding NOAEL of 50 ppm. (1.9 mg/kg/day). A LOAEL could not be identified for females. The NOAEL for females was therefore ≥ 100 ppm (4.9 mg/kg/day).

In a carcinogenicity study in mice (MRID 42208405, 44807220), the relative liver weights increased in males and females by 45% and 30%, respectively, at 1000 ppm compared to the controls, and by 15% in females at 100 ppm after 104 weeks of treatment ($p<0.01$). Microscopic examination showed changes in the liver in males at 1000 ppm (refer to page 12 for details) . No

treatment-related effects were seen at 1, 10, or 100 ppm.. The LOAEL is 100 ppm in the diet (10.72 mg/kg/day for males; 11.72 mg/kg/day for females), based on increased incidences of brown pigmented macrophages in the liver of both sexes, increased incidences of eosinophilic vacuolated hepatocytes in males, and increased liver weights in females. The NOAEL was 10 ppm (1.12 mg/kg/day for males; 1.16 mg/kg/day for females).

In another carcinogenicity study in mice (MRID 44807222, 44807221), there was a significant decrease in survival in females at 7000 ppm fluazinam (control, 52%; 7000 ppm, 24%, $p < 0.01$). All females were terminated after 97 weeks of treatment because of low survival at the high-dose. At termination, relative liver weights 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 changes in the liver (in males at ≥1000 ppm and in females at ≥3000 ppm), the cervical spinal cord (males and marginally in females at ≥3000 ppm). Refer to pages 18 and 19 for details. 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 a chronic oral toxicity study (MRIDs 42270603, 44807219), Fluazinam (Lot No. 8412-20, 95.3% purity) was administered to groups of six male and six female beagle dogs/dose for 52 weeks at doses of 0, 1, 10, or 50 mg/kg/day in gelatin capsules. The most notable clinical signs were increased incidence of salivation and nasal dryness, mainly in the high-dose dogs but nasal dryness was also slightly increased in females at 10 mg/kg/day. The total body weight gain was significantly reduced (29%, $p<0.05$) only in females but was also lower in males (-19%).

Alkaline phosphatase was significantly increased (52-183%; $p<0.05$, 0.01, or 0.001) in high-dose dogs throughout the treatment period. Absolute liver weight (males, 37%; females, 16%; $p<0.05$) and the liver/body weight ratio (males, 45%; females, 47%; $p<0.01$) were increased in high-dose dogs. An increase in liquefied GI tract contents and incidence/severity of stomach mucosal lymphoid hyperplasia was seen in mid- and high-dose dogs of both sexes, although in females, neither incidence nor mean severity of the hyperplasia at these dose levels showed a dose-related increase.

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

5. Mode of Action Studies

There were no mechanistic studies relevant to the induction of tumors in either rats or mice available for review.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

The CARC concluded that:

- **In the combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats (1988), there was some evidence that fluazinam was carcinogenic to male rats** because: 1) There were statistically significant ($p<0.05$) positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/adenocarcinomas. There was also a statistically significant ($p< 0.05$) increase by pair-wise comparison of the high dose group (1000 ppm or 40 mg/kg/day) with the controls for combined follicular cell adenomas/adenocarcinomas (23% vs 8% in controls); 2) The incidences of thyroid gland adenomas at 100 and 1000 ppm (15% and 17%, respectively) and adenocarcinomas at 1000 ppm (6%) were slightly outside their respective ranges in the historical control data (range: adenomas: 0%-13%; adenocarcinomas: 0%-5%). However, the increased incidence of thyroid adenomas at 100 ppm was not observed in a more recent 1993 chronic study. Because of the significant survival disparities between the control and treated groups of male rats, the thyroid tumor data were analysed by the Peto's Prevalence Test. The results revealed a borderline statistically significant ($p= 0.056$) increase by pair-wise comparison of the 1000 ppm dose group with the controls and a statistically significant ($p<0.05$) positive trend, for thyroid follicular cell adenocarcinomas indicating that there was a malignant component to the thyroid tumors; and 3) For combined follicular cell adenomas/adenocarcinomas, Peto's Prevalence Test also showed a statistically significant ($p<0.05$) increase by pair-wise comparison of the high dose male group with the controls (26% vs 9% in controls). At 1000 ppm, there was increased in thyroid weights at 52 weeks, enlarged thyroids and a slightly increased incidence of thyroid gland follicular cell hyperplasia at 104 weeks in males. The CARC noted that animals in the lower dose groups were not microscopically examined for thyroid lesions unless abnormalities were observed in that organ at gross necropsy. Therefore, the percentage incidences of thyroid tumors in these lower dose groups may have been somewhat misleading (too high). There was no treatment-related increase in tumor incidence seen in the female rats in this study.

  The highest dose level tested was considered to be adequate and not excessive because the histopathological changes observed were not severely adverse. There was decrease in body weight gains (up to 15% and 35% in males and females, respectively) and the survival of the animals was not decreased by treatment with the test material. **The CARC concluded that there was some evidence that the thyroid tumors observed in the male rats in this study were treatment-related.**

- **In the carcinogenicity study in CD-1 mice (1988), there was <u>clear evidence</u> that fluazinam was carcinogenic to male mice** because: 1) There were statistically significant ($p<0.05$ or $p<0.01$) positive trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas for the male mice. There were also statistically significant ($p<0.05$) increases by pair-wise comparison of the high dose group (1000 ppm or 107 mg/kg/day) with the controls for hepatocellular adenomas (34% vs 16% in controls) and for hepatocellular carcinomas (34% vs 19% in controls). In addition, there was a statistically significant ($p<0.01$) increase by pair-wise comparison of the high dose group with the controls for combined hepatocellular adenomas/carcinomas (62% vs 33% in controls); and 2) The incidence of hepatocellular adenomas (34%) at the highest dose level was outside the historical control range for 1981-1983 (4-27%) and for 1986-1988 (8-23%). The incidence of hepatocellular carcinomas (34%) at 1000 ppm was also outside the historical control range for 1986-1988 (5-13%), but not for 1981-1983 (12-38%). Among high dose males, several additional histopathological changes were observed in the liver. These changes included increased incidences of basophilic hepatocytes, eosinophilic vacuolated hepatocytes, brown pigmented macrophages and granulomatous hepatitis. Among high dose females, an increased incidence of brown pigmented macrophages was observed in the liver. There were, however, no treatment-related tumors observed in the female mice in this study.

  The highest dose level tested was considered to be adequate and not excessive because liver and brain toxicity observed in the male mice was not considered to be severely adverse. Although there were no significant changes in body weight gain or survival, mean liver weight gains were increased in males and females and histopathological changes were observed in the livers and brain of males and females at 1000 ppm. **The Committee concluded that there was <u>clear evidence</u> of treatment-related increases in both benign and malignant liver tumors in the male mice in this study.**

- **In a recent carcinogenicity study in CD-1 mice (1996), there was <u>equivocal/some evidence</u> that fluazinam was carcinogenic to male mice** because: 1) There were no statistically significant positive trends for hepatocellular adenomas, carcinomas or combined adenomas/carcinomas. At the mid dose (3000 ppm or 377 mg/kg/day), there was a statistically significant ($p<0.01$) 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); however, there was no statistically significant increase in hepatocellular carcinomas by pair-wise comparison with the controls. At the high dose (7000 ppm or 964 mg/kg/day), there was no statistically significant increase by pair-wise comparison with the controls for hepatocellular adenomas or carcinomas when considered separately, but there was a statistically significant ($p<0.05$) increase by pair-wise comparison with the controls for combined adenomas/carcinomas (33% vs 18% in controls). The incidence of combined

hepatocellular adenomas/carcinomas at ≥3000 ppm was driven by the adenomas; and 2) The incidence of hepatocellular adenomas at 3000 ppm (47%) was outside the historical control range for 1991-1993 (8-34%) and for 1987-1993 (0-31%), but the incidence at 7000 ppm (30%) did not exceed the range for the comparable historical controls. Similarly, the incidence of combined hepatocellular adenomas/carcinomas at 3000 ppm (49%) was outside the historical control range for 1987-1993 (4-42%), but the incidence at 7000 ppm (33%) did not exceed the range for the comparable historical controls. The incidences of hepatocellular carcinomas for males at 3000 ppm (9%) and at 7000 ppm (8%) also did not exceed the historical control range for 1991-1993 (2-16%) and for 1987-1993 (4-17%). For the male mice, the tumorigenic response did not occur in a dose-related. manner.

The highest dose level tested for the male mice in this study was considered to be adequate and not excessive. The toxicity observed at this dose level (decreased body weight gain of 32% compared to controls, decreased efficiency of food utilization, and histopathological effects in the liver, brain and spinal cord) was not considered to be life-threatening . In addition, no treatment-related increase in necrosis or hyperplasia was observed in the liver despite an increase in liver weight. This dose level (7000 ppm) is equivalent to the limit dose. **Therefore, the Committee concluded that there was equivocal/some evidence for hepatocarcinogenicity in the male mice in this study because the data suggested a possible treatment-related increase in benign liver tumors. The Committee also noted that the increase in hepatocellular tumors observed in the males in the 1988 mouse study at a dose level of 1000 ppm (107 mg/kg/day) was not confirmed in this 1996 mouse study at the same dose level of 1000 ppm (126 mg/kg/day) using the same strain of mice.**

For female mice, only a statistically significant ($p<0.05$) positive trend was observed for combined hepatocellular adenomas/carcinomas. **The Committee also concluded that the hepatocellular tumors observed in the female mice at**

the highest dose (7000 ppm or 1185 mg/kg/day) 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 increase in mortality for the high-dose females in this study (76% at 7000 ppm vs 48% in controls).

2. Mutagenicity

The CARC concluded that technical grade fluazinam was negative for mutagenic potential in a battery of acceptable mutagenicity studies. These studies included two reverse gene mutation assays in bacteria, two mouse lymphoma forward gene mutation assays, a mammalian cell cytogenetics assay, and a mouse bone marrow micronucleus assay.

3. Structure-Activity Relationships

There are no structurally-related compounds in HED's database.

4. Postulated mode of action

No mechanistic studies related to the mechanism of thyroid tumor induction in rats or mice were available. However, the Committee considered various factors in making the determination of whether neoplasms are due to thyroid-pituitary imbalance. These included increases in cellular growth *in vivo*; hormone changes; site of action; dose correlations; reversibility; lesion progression; structure-activity analysis and other studies. In addition, the Committee also gave consideration to the extent to which genotoxicity may account for the observed tumor effects, the dose-response and the occurrence of tumors in other tissues in addition to the thyroid (Refer to Budd, 2000 for evidence demonstrating antithyroid activity).

Following the evaluation of evidence for antithyroid activity, the CARC determined that there was some evidence of increases in cellular growth (increased thyroid weights, enlarged thyroids and increased incidences of thyroid follicular cell hyperplasia) in the rat studies. The increases in thyroid tumors were only observed at these dose levels. Thus, no dose-response was observed. There was very limited evidence of progression from thyroid hypertrophy/hyperplasia to neoplasia in rats. The presence of a second tumor type (liver) in addition to the thyroid was not demonstrated in rats. The results of mutagenicity assays were negative suggesting that mutagenicity does not play a role in the tumorigenic activity for this chemical. Although the mutagenicity data were negative, the Committee concluded that the criteria for a threshold effect were largely not met for the following reasons: there was no study that evaluated the effect of fluazinam on TSH , T4 and T3 levels; the reversibility of thyroid hormone effects was not demonstrated. No determinations were made in any of the available studies on microsomal mixed function oxidase activity in liver tissues. In addition, no special thyroid function studies were conducted. **The Committee concluded that there are insufficient data to determine whether the thyroid tumors in the rat associated with administration of fluazinam are due to a disruption in thyroid-pituitary homeostasis.**

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July, 1999), the CARC classified fluazinam into the category **"Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"** based on the following weight-of-the-evidence considerations:

1. There was some evidence that fluazinam induced an increase in thyroid gland follicular cell tumors in male rats, but not in female rats. In one study in mice, there was clear

evidence that an increased incidence of hepatocellular tumors observed in the male mice was treatment-related. In another study in mice, there was equivocal/some evidence that fluazinam may have induced an increase in hepatocellular tumors in the male mice. Increases in hepatocellular tumors observed in the female mice in the latter study were not statistically significant and some occurred at an excessively toxic dose level. The thyroid gland follicular cell tumors of concern were seen only in male rats and the hepatocellular tumors of concern were seen only in male mice.

2. Fluazinam was negative in a battery of acceptable mutagenicity assays.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

Not required.

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