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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: February 13, 2001

MEMORANDUM

SUBJECT: **FLUAZINAM** - Report of the Hazard Identification Assessment Review Committee.

FROM: Edwin R. Budd, M.S.
Registration Action Branch 2
Health Effects Division (7509C) *Edwin R. Budd*
2/14/01

THROUGH: Jess Rowland, Co-Chair *Jess Rowland* *2/14/01*
and
Elizabeth Doyle, Co-Chair *E. A. Doyle*
Hazard Identification Assessment Review Committee
Health Effects Division (7509C) *2/14/01*

TO: William Cutchin, Risk Assessor
Registration Action Branch 2
Health Effects Division (7509C)

PC Code: 129098

On October 24, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for fluazinam with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to fluazinam was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996. The conclusions drawn at this meeting are presented in this report.



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Committee Members in Attendance

Members present were: Bill Burnam, Elizabeth Mendez, David Nixon, Jess Rowland, Yung Yang, Jonathan Chen, Brenda Tarplee (Executive Secretary)

Member(s) in absentia: Beth Doyle, Pamela Hurley, Ayaad Assaad

Data evaluation prepared by: Edwin R. Budd, M.S., Registration Action Branch 2

Also in attendance were: Abdallah Khasawinah, Nader Tadayan

Data Evaluation / Report Presentation

Edwin R. Budd 1/14/01
Edwin R. Budd, M.S.
Toxicologist

1. INTRODUCTION

Fluazinam, also known as B-1216, IKF-1216, PP192 and as Frowncide, is a new (not registered) 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.

The end-use formulation proposed for registration is OMEGA 500F (EPA File Symbol 71512-R) which is a flowable liquid concentrate containing 40.0% a.i. (equivalent to 4.17 lb a.i./gallon). It is anticipated that OMEGA 500F may be applied by ground or air. The applicant is ISK Biosciences Corporation, Mentor, Ohio.

In 1992, in response to a request for an Experimental Use Permit (EUP) and a temporary tolerance for fluazinam on peanuts, the Health Effects Division (HED) RfD/Peer Review Committee met to evaluate data submitted in support of the request. At the meeting, the committee concluded that a Provisional Reference Dose could be established based on a NOEL of 3.8 mg/kg/day from a 90-day subchronic feeding study in rats. Using an uncertainty factor of 1,000, the Provisional Reference Dose was calculated to be 0.004 mg/kg/day. See memorandum dated September 29, 1992; Tox Doc No. 013552.

In 1995, the applicant reported the occurrence of vacuolation in the white matter of the brain and spinal cord of mice in an ongoing carcinogenicity study in mice. In response to the report, HED recommended that no further granting of EUPs for fluazinam be approved until the mouse study is reviewed in its entirety and the rat and dog chronic feeding studies are evaluated for potential neurotoxic responses to fluazinam. See memorandum dated August 19, 1995; Tox Doc No. 011647.

More recently, the applicant has completed a full toxicology data base for fluazinam, including an evaluation of the vacuolation of the white matter in the brain and spinal cord of mice, rats and dogs. All the currently available toxicology data on fluazinam has now been reviewed.

On October 24, 2000, the Health Effects Division (HED) Hazard Identification Assessment Review Committee (HIARC) reviewed the recommendations of the toxicology reviewer for fluazinam with regard to the acute and chronic Reference Doses (RfDs) and the toxicological endpoint selection for occupational/residential exposure risk assessments. The potential for increased susceptibility of infants and children from exposure to fluazinam was also evaluated as required by the Food Quality Protection Act (FQPA) of 1996.

2. HAZARD IDENTIFICATION

1. Acute Reference Dose (RfD)

2.1.1 Females 13-50 Years

Study Selected: Developmental toxicity study in rabbits

§ 870.3700

MRID No.: 42248616

Executive Summary: In a developmental toxicity study (MRID 42248616), 16-18 presumed pregnant New Zealand White rabbits per group were administered Fluazinam (95.3% a.i, Lot No.: 8412-20) by gavage in 1% w/v aqueous methyl cellulose at doses of 0, 2, 4, 7 and 12 mg/kg/day on gestation days (GD) 6-19, inclusive. Controls were treated with 1% w/v aqueous methyl cellulose (vehicle). On GD 29, all surviving does were sacrificed and necropsied and all fetuses were weighed, and examined for external malformation/variations. Each fetus was examined viscerally by fresh dissection and the sex determined. All carcasses were eviscerated and processed for skeletal examination.

At 12 mg/kg/day, statistically significant reductions in body weight gain during treatment (0.0 kg vs +0.25 kg for controls on GD 10-20), decreased food consumption (268 g/animal/day vs 368 g/animal/day for controls on GD 6-19) and an increased incidence of liver histopathology (cellular hypertrophy, single cell necrosis, binucleate hepatocytes, increased brown pigment deposition, apoptosis) were considered to be treatment-related. At 7 mg/kg/day, slightly depressed food consumption (139 g/animal/day vs 186 g/animal/day for controls on GD 13-19) and an increased incidence of liver histopathology (cellular hypertrophy, single cell necrosis, binucleate hepatocytes, increased brown pigment deposition, apoptosis) were also considered to be treatment-related. **The maternal toxicity LOAEL is 7 mg/kg/day based on decreased food consumption and increased liver histopathology. The maternal toxicity NOAEL is 4 mg/kg/day.**

At 12 mg/kg/day, an increased incidence of total litter resorptions (0, 0, 0, 1 and 5 for the 0, 2, 4, 7 and 12 mg/kg/day groups respectively) was considered to be treatment-related. Several abortions were also observed (0, 0, 2, 2 and 1 for the 0, 2, 4, 7 and 12 mg/kg/day groups respectively). The total number of litters lost was such that the numbers of litters born were 15, 13, 10, 10 and 7 for the 0, 2, 4, 7 and 12 mg/kg/day groups respectively. Also at 12 mg/kg/day, there was an increased incidence of placental anomalies (0.7, 3.2, 0.0, 0.0 and 18.2 percent fetal incidence for the 0, 2, 4, 7 and 12 mg/kg/day groups respectively) and a slight increase in some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae, and abnormalities of head bones. **The developmental toxicity LOAEL is 12 mg/kg/day based on an increased incidence of total litter resorptions and a possibly increased incidence of fetal skeletal abnormalities. The developmental toxicity NOAEL is 7 mg/kg/day.**

This developmental toxicity study in rabbits is classified **Acceptable/Guideline** and **satisfies** the Subdivision F guideline requirement for a developmental toxicity study in rabbits [OPPTS 870.3700 (OPP 83-3b)]. No major deficiencies were noted in this study.

Dose and Endpoint for Establishing RfD: 7 mg/kg/day. Developmental toxicity NOAEL of 7 mg/kg/day based on increased incidence of total litter resorptions and a possibly increased incidence of fetal skeletal abnormalities (including kinked tail tip, fused or incompletely ossified sternbrae, and abnormalities of head bones) at 12 mg/kg/day (LOAEL).

Uncertainty Factor (UF): 100, based on 10 for intraspecies variation and 10 for interspecies extrapolation.

Comments about Study: In the original DER for this study (HED Doc. No. 009608, 7/13/92), this study was classified as Core-Minimum. However, when the HED RfD/Peer Review Committee met on 8/6/92, the study was downgraded to Core Supplementary because the committee felt that there were inadequate litter numbers at the highest 3 doses and that the NOEL therefore could not be determined. In addition, the committee generally questioned the conduct of the study noting the relatively large number of animals which died prior to or during the study, the presence of significant lung infections, and evidence of general liver alterations which might impact on the metabolism of the test material. The committee required that a new study be performed by the registrant using the same dose levels but with enough animals at the highest 3 doses to ensure at least 12 litters per dose (HED Doc. No. 009727, 9/14/92). Subsequently, at the request of Toxicology Branch I (TB1), the HED RfD/Peer Review Committee met again on 11/19/92 to reconsider its prior position on this study. In support of its request, TB1 submitted a memorandum (dated 11/4/92, copy in HED Doc. No. 013551) providing a rationale as to why, in the opinion of TB1, there was insufficient justification for repeating the study and that a second study would provide little additional information. The following is quoted from the report of the RfD/Peer Review Committee on its second meeting (HED Doc. No. 013551, 3/16/93):

“After thorough consideration of all the issues involved, the Committee felt that although the study was deficient, it was marginally acceptable for regulatory purposes. The Committee noted that an additional dose level, beyond that required by the Guidelines, was included in this study. Furthermore, when the preliminary (range finding) and main studies were considered together, maternal deaths appeared to be randomly distributed between groups.

Therefore, the Committee considered that the repeat of the study at this time was unlikely to provide additional useful information, and the “no-observable effect level” for maternal toxicity (4 mg/kg/day) and for developmental and fetotoxicity (7 mg/kg/day) were unlikely to change in a new study.”

Comments about Endpoint/Uncertainty Factor: The committee considered the developmental toxicity effects observed at the LOAEL in this study (increased incidence of total litter

resorptions and a possibly increased incidence of fetal skeletal abnormalities, including kinked tail tip, fused or incompletely ossified sternebrae, and abnormalities of head bones) to be effects that could occur after a single dose of fluazinam. Also, with respect to developmental toxicity effects, the rabbit is more sensitive than the rat. The NOAEL for developmental toxicity effects in the developmental toxicity study in rats (MRID 42248613) was 50 mg/kg/day and the LOAEL was 250 mg/kg/day.

Acute RfD (females, 13-50 years)	=	$\frac{7 \text{ mg/kg/day (NOAEL)}}{100 \text{ (UF)}}$	=	0.07 mg/kg
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2.1.2 General Population (including infants and children)

Study Selected: Acute neurotoxicity study in rats

§ 870.6200

MRID No.: 44807210

Executive Summary: In an acute oral neurotoxicity study (MRID 44807210), single gavage doses of 0, 50, 1000 or 2000 mg/kg fluazinam (96.8%, Lot No.: 1030/91) in 1.5% (w/v) aqueous methylcellulose were administered to groups of fasted Sprague-Dawley rats (10/sex/dose). Functional Observational Battery (FOB) and Motor Activity (MA) assessments were performed before test substance administration, between 5 and 7 hours post-administration (time of peak effect) and on days 7 and 14. Body weights were measured weekly and clinical signs were recorded daily. The animals were sacrificed and grossly examined 14 days after administration of the test material. Five rats/dose/sex were perfused *in situ* for neuropathological evaluation.

There were no treatment-related deaths, clinical signs, or body weight effects during the study. Treatment-related soft stools were observed in mid- and high-dose males and females, but only on the day of treatment. Treatment-related mean motor activity values were significantly decreased (23-65%) in mid- and high-dose females compared to controls (not dose-related), but only on the day of treatment. No other treatment-related effects of any kind were observed at any time during the study. No treatment-related gross effects or histopathology were observed. Since the decreased mean motor activity values were observed in one sex only (females), on one occasion only (on the day of dosing), at high doses only (≥ 1000 mg/kg), and were not dose-related, and were observed in one study only (not observed in the subchronic neurotoxicity study in rats, MRID 44807217, 44807218), it is likely that this treatment-related effect (decreased mean motor activity values in females only on the day of dosing only) is a manifestation of acute general systemic toxicity and not a direct neurotoxic response to the administration of the test material.

Under the conditions of this study, the acute general systemic toxicity LOAEL is 1000 mg/kg for male and female rats based on soft stools and decreased motor activity. The acute general systemic toxicity NOAEL is 50 mg/kg for male and female rats. The LOAEL for neurotoxic effects is not identified (> 2000 mg/kg). The NOAEL for neurotoxic effects is 2000 mg/kg.

This acute oral neurotoxicity study is classified **Acceptable/ Guideline**. This study does satisfy the guideline requirement for an acute oral neurotoxicity study [OPPTS 870.6200 (81-8ss)] in rats.

Dose and Endpoint for Establishing RfD: 50 mg/kg. Acute general systemic toxicity NOAEL of 50 mg/kg for male and female rats based on soft stools and decreased motor activity at 1000 mg/kg (LOAEL) .

Uncertainty Factor(s): 100, based on 10 for intraspecies variation and 10 for interspecies extrapolation.

Comments about Study/Endpoint/Uncertainty Factor: Due to the large dose spread in this study between the NOAEL (50 mg/kg) and the LOAEL (1000 mg/kg), the true NOAEL in this study is probably much higher than 50 mg/kg. This study, however, provides the best data available for determining an acute RfD for the general population (including infants and children).

<p>Acute RfD (general population, incl. infants and children)</p>	<p>=</p>	<p><u>50 mg/kg (NOAEL)</u> 100 (UF)</p>	<p>=</p>	<p>0.50 mg/kg</p>
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2. Chronic Reference Dose (RfD)

Study Selected: 2-Year carcinogenicity study in mice (co-critical study #1) § 870.4200

MRID No.: 42208405, 44807220, 44807212

Executive Summary: In an oncogenicity study (MRID 42208405, 4807220, 44807212), Fluazinam (95.3% a.i., lot no. 8412-20) was administered to groups of 52 male and 52 female CD@-1 mice in the diet at concentrations of 0, 0, 1, 10, 100, or 1000 ppm. There were 2 control groups. The test diets were given for 104 weeks. These concentrations resulted in mean daily compound intakes of 0.12, 1.12, 10.72, and 107 mg/kg/day for 1 ppm, 10 ppm, 100 ppm, and 1000 ppm, respectively, for males and 0.11, 1.16, 11.72, and 117 mg/kg/day, respectively, for females. Additional microscopic review of brain and spinal cord was presented in MRID 44807220. A four-week-range finding study (MRID 44807212) using 0, 10, 50, 250, or 3000 ppm in the diet was also conducted.

Treatment with Fluazinam did not result in treatment-related changes in survival, clinical signs, body weights, body weight gains, food consumption or hematology parameters. The group mean liver weights adjusted for body weight were increased in males and females by 45% and 30%, respectively, at 1000 ppm compared to the controls, and by 15% in females at 100 ppm after 104 weeks of treatment ($p < 0.01$). Microscopic examination showed increased incidences of liver areas containing basophilic hepatocytes (controls, 12%; 1000 ppm, 38%, $p < 0.01$) and/or eosinophilic vacuolated hepatocytes (controls, 1%; 100 ppm, 8%, $p < 0.05$; 1000 ppm 19%, $p < 0.01$) in treated males compared to the controls. Increased incidences of granulomatous hepatitis of minimal severity were seen in high-dose males (controls, 11%; 1000 ppm, 25%, $p < 0.01$) and females (controls, 11%; 1000 ppm, 21%, $p < 0.01$). Higher incidences of aggregates of brown pigmented macrophages were seen in the livers of treated males (controls, 13%; 100 ppm, 27%, $p < 0.05$; 1000 ppm, 62%, $p < 0.01$) and females (controls, 15%; 1 ppm, 40%, $p < 0.01$; 10 ppm, 21%, NS; 100 ppm, 38%; 1000 ppm, 50%, $p < 0.01$). Granulomatous hepatitis and brown pigmented macrophage aggregates were most commonly seen in mice that survived until the end of the study. The only effects that were not associated with the liver were an increased incidence of thymic hyperplasia in high-dose females (controls, 5%; 1000 ppm, 21%, $p < 0.01$), and increased incidences of cystic thyroid follicles in high-dose males (controls, 23%; 1000 ppm, 52%, $p < 0.01$) and high-dose females (controls, 16%; 1000 ppm, 33%, $p < 0.01$).

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

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

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

slightly exceeded the upper range of historic controls (27%). The dosing was adequate for an oncogenicity study based on the liver and brain toxicity at 1000 ppm.

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

Study Selected: 1-Year chronic oral study in dogs (co-critical study #2) § 870.4100

MRID No.: 42270603, 44807219

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

No animals died as a result of treatment. The most notable clinical signs were increased incidence of salivation and nasal dryness, mainly in the high-dose dogs but nasal dryness was also slightly increased in females at 10 mg/kg/day. Body weight was mildly decreased at high dose (-4%, males and -9%, females; not analyzed statistically), and total body weight gain was significantly reduced (29%, $p < 0.05$; -13% when calculated as a percentage of initial body weight) only in females but was also lower in males (-19%; -9% as a percentage of initial body weight). Hematocrit, hemoglobin, and RBC counts of high-dose dogs were consistently lower (8-17%; $p < 0.05$, 0.01, or 0.001) than controls throughout the treatment period, and WBC counts were elevated (32-64%, $p < 0.05$ or 0.001) at study end (these findings considered treatment-related but not biologically significant). Alkaline phosphatase was significantly increased (52-183%; $p < 0.05$, 0.01, or 0.001) in high-dose dogs throughout the treatment period.

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

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

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

Dose and Endpoint for Establishing RfD: 1.1 mg/kg/day, 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 at 10.72 mg/kg/day (LOAEL).

Uncertainty Factor(s): 100, based on 10 for intraspecies variation and 10 for interspecies extrapolation.

Comments about Studies/Endpoints/Uncertainty Factor: This 2-year carcinogenicity study in mice, rather than the 1-year chronic oral study in dogs, was used to establish the RfD because the treatment-related effects at the LOAEL in the mouse study were related to liver toxicity (the regularly observed target organ for fluazinam in many studies), whereas the effects at the LOAEL in the dog study (increased incidence of nasal dryness in females and increased incidence/severity of gastric lymphoid hyperplasia in males and females) were unrelated to liver toxicity. It was noted by the committee that the NOAELs in the mouse study (1.12 mg/kg/day in males and 1.16 mg/kg/day in females) and in the dog study (1 mg/kg/day in males and females) and the LOAELs in the mouse study (10.72 mg/kg/day in males and 11.72 mg/kg/day in females) and in the dog study (10 mg/kg/day in males and females) were quite similar.

In addition, the committee observed that a treatment-related neurotoxic lesion described as vacuolation of the white matter of the central nervous system (CNS) was observed in both the 2-year carcinogenicity study in mice (at 107/117 mg/kg/day, but not at 10.72/11.72 mg/kg/day in M/F) and in the 1-year chronic oral study in dogs (at 50 mg/kg/day, but not at 10 mg/kg/day in M/F). The committee particularly noted that the dose level at which vacuolation of the CNS was not observed in the 2-year mouse study (neurotoxic NOAEL) was 10.72/11.72 mg/kg/day in M/F and was 10 fold higher than the NOAEL of 1.12/1.16 mg/kg/day in M/F for general systemic toxicity in the same study. Similarly, the dose level at which vacuolation of the CNS was not observed in the 1-year dog study (neurotoxic NOAEL) was 10 mg/kg/day in M/F and was also 10 fold higher than the NOAEL of 1 mg/kg/day in M/F for general systemic toxicity in the same study. It was later determined in a series of special mechanistic studies that this CNS lesion was induced solely by an impurity (Impurity-5) in technical grade fluazinam and not by fluazinam per se. Impurity-5 was present in the various lots of technical grade fluazinam used in toxicity studies at highly variable concentrations up to 0.20%. In the 2-year carcinogenicity study in mice (MRID 42208405, 44807220, 44807212), the calculated intake of Impurity-5 at the dose level of 107/117 mg/kg/day in M/F was 0.22 mg/kg/day, at which level vacuolation of the CNS was observed in this study. In this same study, the calculated intake of Impurity-5 at the dose level of 10.72/11.72 mg/kg/day in M/F was 0.022 mg/kg/day, at which level vacuolation of the CNS was not observed in this study. In the 1-year chronic oral study in dogs (MRID 42270603, 44807219), the calculated intake of Impurity-5 at the dose level of 50 mg/kg/day in M/F was 0.1 mg/kg/day, at which level vacuolation of the CNS was observed in this study. In this same study, the calculated

intake of Impurity-5 at the dose level of 10 mg/kg/day in M/F was 0.02 mg/kg/day, at which level vacuolation of the CNS was not observed in this study. The similar dose levels of Impurity-5 at which vacuolation of the CNS was observed (and not observed) in these mouse and dog studies also supports these studies being co-critical with one another with respect to determining the chronic RfD for fluazinam. See the discussion under 5.2 Neurotoxicity later in this document for a more detailed and complete discussion of vacuolation of the CNS observed in some of the toxicity studies on fluazinam.

The committee also noted that a 2-year chronic feeding/carcinogenicity study in rats (MRID 42248620, 44807223) is available which has an NOAEL of 0.38 mg/kg/day for males and of 0.47 mg/kg/day for females, which is lower than the NOAEL (1.1 mg/kg/day) selected for establishing the chronic RfD. The reason for not selecting the NOAEL from this study to establish the chronic RfD is that the next highest dose level tested in this study was 3.8 mg/kg/day in males and 4.9 mg/kg/day in females (a 10 fold higher dose); that a second 2-year chronic feeding/ carcinogenicity study in rats (MRID 44839901, 44807213) subsequently was performed with 2 dose levels intermediate between the dose levels of 0.38/0.47 mg/kg/day and 3.8/4.9 mg/kg/day used in the first study; and that the NOAEL observed in the second study was 1.9 mg/kg/day for males and 4.9 mg/kg/day for females, which is higher than the NOAEL (1.1 mg/kg/day) selected for establishing the chronic RfD.

<p>Chronic RfD (general population, incl. infants and children)</p>	<p>=</p>	<p><u>1.1 mg/kg/day (NOAEL)</u> 100 (UF)</p>	<p>=</p>	<p>0.011 mg/kg/day</p>
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3. Occupational/Residential Exposure

2.3.1 Short-Term (1 - 7 days) Incidental Oral Exposure

Study Selected: Developmental toxicity study in rabbits § 870.3700

MRID No.: 42248616

Executive Summary: See Executive Summary for Acute Reference Dose (RfD), Females 13-50 Years.

Dose and Endpoint for Risk Assessment: 4 mg/kg/day. Maternal toxicity NOAEL of 4 mg/kg/day, based on decreased food consumption and an increased incidence of liver histopathological lesions (cellular hypertrophy, single cell necrosis, binucleate hepatocytes, increased brown pigment deposition and apoptosis) at 7 mg/kg/day (LOAEL).

Comments about Study/Endpoint: There are no residential uses for fluazinam proposed at this time. This endpoint was selected for future uses as may be needed. The endpoints of concern are appropriate for this exposure scenario and population of concern (children).

2.3.2 Intermediate-Term (1-Week to Several Months) Incidental Oral Exposure

Study Selected: Developmental toxicity study in rabbits (co-critical study #1) § 870.3700

MRID No.: 42248616

Executive Summary: See Executive Summary for Acute Reference Dose (RfD), Females 13-50 Years.

Dose and Endpoint for Risk Assessment: 4 mg/kg/day. Maternal toxicity NOAEL of 4 mg/kg/day, based on decreased food consumption and an increased incidence of liver histopathological lesions (cellular hypertrophy, single cell necrosis, binucleate hepatocytes, increased brown pigment deposition and apoptosis) at 7 mg/kg/day (LOAEL).

Comments about Study/Endpoint: There are no residential uses for fluazinam proposed at this time. This endpoint was selected for future uses as may be needed. The endpoints of concern are appropriate for this exposure scenario and population of concern (children).

Results from the following study are considered to be co-critical for the dose and endpoint selected above for intermediate-term incidental oral exposure.

Study Selected: 90-Day feeding study in rats (co-critical study #2) § 870.3100

MRID No.: 42248610, 44807214

Executive Summary: In a subchronic oral toxicity study (MRID 42248610, 44807214), technical grade fluazinam (98.5% a.i.) was administered in the diet to 10 CD (remote Sprague-Dawley strain) 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). Slides of brain and cervical spinal cord from all control and 500 ppm rats were later re-examined to assess for vacuolation of the white matter in the central nervous system (MRID 44807214).

No treatment-related mortalities, clinical signs of toxicity, changes in body weights or body weight gains, differences in food or water consumption, or ophthalmological findings were observed. No treatment-related effects in hematology, clinical chemistry, or urinalyses parameters were noted. Gross necropsies were negative. At termination, statistically significant treatment-related increases were observed in the liver of 500 ppm males (absolute weights increased 8 % (not significant) and relative liver/body weight ratios increased 11% in comparison to controls), in the lungs of 500 ppm females (absolute weights increased 18 % and relative lung/body weight ratios increased 25% in

comparison to controls), and in the uterus of 500 ppm females (absolute weights increased 36 % and relative uterus/body weight ratios increased 43% in comparison to controls). 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). There was no effect of treatment on the incidence or severity of vacuolation of the white matter of the brain or cervical spinal cord in the 500 ppm rats as compared with the controls.

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, increases in absolute and relative lung and uterus weights in females, and increases in histopathology in the liver of males (increased incidences of periacinar hepatocyte hypertrophy and sinusoidal chronic inflammation).

This subchronic oral toxicity study in rats is classified **Acceptable/guideline** and satisfies the Subdivision F guideline requirement for a subchronic oral toxicity study [OPPTS 870.3100 (§82-1a) in rats. No major deficiencies were noted in this study.

Dose and Endpoint: 3.8 mg/kg/day in males and 4.3 mg/kg/day in females. The NOAEL in this study was 3.8 mg/kg/day in males and 4.3 mg/kg/day in females. At the LOAEL of 38 mg/kg/day in males and 44 mg/kg/day in females, increases in absolute and relative liver weights in males, increases in absolute and relative lung and uterus weights in females, and increases in histopathology in the liver of males (increased incidences of periacinar hepatocyte hypertrophy and sinusoidal chronic inflammation) were observed.

2.3.3 Dermal Absorption

Comments about Dermal Absorption: There is no dermal absorption study available on fluazinam. A dermal absorption factor was estimated by comparing the LOAEL from a 21-day dermal toxicity study in rats to the LOAEL from a 4-week range-finding feeding study in rats based on a common endpoint (liver toxicity).

Selected Study #1: 21-Day dermal toxicity study in rats § 870.3200

MRID No.: 42270602

Systemic NOAEL = 10 mg/kg/day

Systemic LOAEL = 100 mg/kg/day, based on increased aspartate aminotransferase (AST) and increased cholesterol levels in males

Selected Study #2: 4-Week range-finding feeding study in rats § none

MRID No.: 44807213

NOAEL = 5.1 mg/kg/day (males)
 5.3 mg/kg/day (females)
 LOAEL = 26.4 mg/kg/day (males)
 25.9 mg/kg/day (females), based on decreased body weight gain and decreased food consumption in females, increased serum phospholipids in females, increased total cholesterol in males and females, increased relative liver weights in females, and liver histopathological effects (periacinar hypertrophy) in males

$$\text{Estimated Dermal Absorption Factor} = \frac{\text{Oral LOAEL} \times 100}{\text{Dermal LOAEL}} = \frac{25 \text{ mg/kg/day} \times 100}{100 \text{ mg/kg/day}} = 25\%$$

2.3.4 Short-term Dermal (1 - 7 days) Exposure

Study Selected: 21-Day dermal toxicity study in rats § 870.3200

MRID No.: 42270602

Executive Summary: In a 21-day repeated dose dermal toxicity study (MRID 42270602), groups of 10 male and 10 female CD (Sprague-Dawley) rats were treated with Fluazinam technical (98.0% a.i.; lot no. 8303-2) in 0.5% methylcellulose in distilled water at doses of 0, 10, 100 or 1000 mg/kg/day. Animals were treated by dermal occlusion for 6 hours per day, 7 days per week, for 3 weeks.

No treatment-related mortalities occurred. At 1000 mg/kg/day, decreased body weight gain in males (19% compared to controls, $p < 0.05$) was observed. Liver damage in both males and females was also evident at 1000 mg/kg/day as demonstrated by increased absolute liver weights (17-26%), increased relative liver/body weight ratios (27-30%), statistically significant increases in aspartate aminotransferase (AST) and cholesterol levels, and highly increased incidences of periacinar hepatocellular hypertrophy in males and females. At 100 mg/kg/day, statistically significant increases in AST and cholesterol levels were observed in males. **The LOAEL for systemic toxicity is 100 mg/kg/day based on increased AST and increased cholesterol levels in males. The NOAEL for systemic toxicity is 10 mg/kg/day.**

At 1000 mg/kg/day, slight to severe erythema and atonia were observed after 11-13 days and encrustation and/or staining at 21 days in males and females. At 100 mg/kg/day, slight erythema was observed after 14 days in males and females and encrustation and/or staining at 21 days in females. At 10 mg/kg/day, slight erythema was noted after 13 days in one male. Histologically, at 1000 mg/kg/day and 100 mg/kg/day, acanthosis, dermatitis, scabs and ulceration were noted in males and females. At 10 mg/kg/day, acanthosis and dermatitis were observed in males and females. At 10 mg/kg/day, the test material was considered to be a very mild irritant. **The LOAEL for dermal toxicity is ≤ 10 mg/kg/day based on erythema, acanthosis and dermatitis**

in males and/or females. No NOAEL for dermal toxicity was determined in this study (< 10 mg/kg/day).

This 21-day dermal toxicity study in rats is classified **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a 21/28-day dermal toxicity study [OPPTS 870.3200 (OPP 82-2)]. No major deficiencies were noted in this study.

Dose and Endpoint for Risk Assessment: 10 mg/kg/day. Systemic toxicity NOAEL of 10 mg/kg/day, based on increased aspartate aminotransferase (AST) and increased cholesterol levels in males at 100 mg/kg/day (LOAEL).

Comments about Study/Endpoint: The hazard identified is from a dermal study which is appropriate for this exposure (dermal) and duration of concern. Also, the developmental effects in rats were seen at a higher dose (at 250 mg/kg/day) compared to the systemic toxicity seen via the dermal route (at 1000 mg/kg/day).

2.3.5 Intermediate-term Dermal (1-Week to Several Months) Exposure

Study Selected: 21-Day dermal toxicity study in rats § 870.3200

MRID No.: 42270602

Executive Summary: See 2.3.4 Short-term Dermal (1-7 days) Exposure

Dose and Endpoint for Risk Assessment: 10 mg/kg/day. Systemic toxicity NOAEL of 10 mg/kg/day, based on increased aspartate aminotransferase (AST) and increased cholesterol levels in males at 100 mg/kg/day (LOAEL).

Comments about Study/Endpoint: See Short-term Dermal.

2.3.6 Long-term Dermal (Several Months to Lifetime) Exposure

The 2-year carcinogenicity study in mice and the 1-year chronic oral study in dogs, co-critical studies used to determine the chronic RfD, were recommended to evaluate the long-term dermal hazard of fluazinam.

Study Selected: 2-Year carcinogenicity study in mice (co-critical study #1) § 870.4200

MRID No.: 42208405, 44807220, 44807212

Executive Summary: See 2.2 Chronic Reference Dose (RfD)

Study Selected: 1-Year chronic oral study in dogs (co-critical study #2) § 870.4100

MRID No.: 42270603, 44807219

Executive Summary: See 2.2 Chronic Reference Dose (RfD)

Dose and Endpoint for Risk Assessment: 1.1 mg/kg/day, 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 at 10.72 mg/kg/day (LOAEL).

Comments about Study/Endpoint: This dose and endpoint were also used for establishing the RfD. See 2.2 Chronic Reference Dose (RfD).

2.3.7 Inhalation Exposure (All Durations)

Study Selected: 7-Day range-finding inhalation study in rats § none
(test material: Frownicide® WP, containing 51.9% fluazinam)

MRID No.: 42248621

Executive Summary: In a 7-day range-finding inhalation toxicity study (MRID 42248621), groups of five male and five female young adult CD rats were exposed nose-only to Frownicide® WP (51.9% Fluazinam, a.i., Batch No. 004) for two 3-hour periods per day for 7 days at concentrations of 0, 0.003, 0.011, 0.032, or 0.110 mg/L. The estimated achieved dosages of Frownicide® WP over the 7 days of treatment were calculated to be 0.72, 2.76, 7.93 and 27.43 mg/kg/day for males and 0.75, 2.97, 8.50 and 29.23 mg/kg/day for females for the concentrations of 0, 0.003, 0.011, 0.032, and 0.110 mg/L, respectively. The mass median aerodynamic diameter (MMAD) was estimated to be 3.22-3.98 µm and the geometric standard deviation was 2.04-2.69 µm. Approximately 60-70% of particles had an aerodynamic diameter < 6.0 µm. The animals were observed daily. Hematology, clinical chemistries and urinalyses were performed. All animals were necropsied after completion of exposure, but no histopathology was performed.

No rats died during the study. No clinical signs of toxicity were noted from any rat. The body weight changes of all groups were similar to that of the control group. No toxicologically significant effects of the test material were noted on food consumption, water consumption, food efficiency, hematology, clinical chemistries, or urinalyses. At 0.110 mg/L, slightly increased lung weights (males and females), slightly increased testes weights (males), and slightly increased liver weights (females) were observed. At 0.032 mg/L, slightly increased testes weights (males), and slightly increased liver weights (females) were also observed. No macroscopic changes attributed to treatment with test material were noted at necropsy. Histopathological examination of tissues was not performed.

The LOAEL is 0.032 mg/L (7.93 mg/kg/day in males and 8.50 mg/kg/day in females), based on slightly increased testes weights (males) and slightly increased liver weights (females). The NOAEL is 0.011 mg/L (2.76 mg/kg/day in males and 2.97 mg/kg/day in females).

This inhalation study is classified as **Acceptable/Non-guideline**. It does not satisfy the subdivision F guideline requirements for a repeated dose inhalation study in the rat because histopathological examination of tissues was not performed. The study was conducted as a range-finding study (for a four-week inhalation study with Frowncide® WP in rats) and is acceptable for that purpose.

Dose and Endpoint for Risk Assessment: 1.38 mg/kg/day, based on slightly increased testes weights (males) and slightly increased liver weights (females) at the LOAEL of 0.032 mg/L (7.93 mg/kg/day in males and 8.50 mg/kg/day in females).

Comments about Study/Endpoint: The inhalation dose of 1.38 mg/kg/day selected to evaluate the inhalation risks of fluazinam is protective of developmental effects, where the NOAEL for developmental toxicity was 7 mg/kg/day in the developmental toxicity study in rabbits (MRID 42248616). When feasible, the inhalation route of exposure is the most appropriate route to use for evaluating inhalation risk. Finally, it is noted that the target organ in the 7-day range-finding inhalation study (liver) and in many other studies on fluazinam consistently is the liver.

The adjustment to the NOAEL in this study is appropriate in order to determine the dose for evaluating the inhalation hazard of fluazinam. Since the test material in this study was not technical grade fluazinam, but rather was Frowncide® WP (approximately 50% fluazinam), the NOAEL from this study (0.011 mg/L or 2.76 mg/kg/day in males and 2.97 mg/kg/day in females) should be reduced by half to account for this (i.e. adjusted NOAEL = 1.38 mg/kg/day for males and 1.48 mg/kg/day for females). Also, it should be recalled that no histopathology was performed in this study and therefore the true NOAEL may be lower than that demonstrated in the study.

For evaluation of short-term (1-7 days) inhalation exposure, an extra factor of 3X should be applied to the conventional uncertainty factor of 100X to account for the lack of histopathology in the inhalation study. MOE = 300.

For evaluation of intermediate-term (7 days to several months) and long-term (several months to life-time) inhalation exposure, an extra factor of 10X should be applied to the conventional uncertainty factor of 100X to account for the lack of histopathology and the use of a short-term (7 days) study to evaluate intermediate-term and long-term inhalation exposure. MOE = 1000.

The HIARC determined that a 28-day inhalation study in rats is a data gap and should be required to support the registration of fluazinam.

2.3.8 Margins of Exposure for Occupational/Residential Risk Assessments

There are no residential uses for fluazinam at the present time.

For short-term and intermediate-term incidental oral exposure risk assessments, a MOE of 100 is required. This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation).

For all dermal exposure risk assessments, a MOE of 100 is required. This is based on the conventional uncertainty factor of 100X (10X for intraspecies extrapolation and 10X for interspecies variation).

For short-term inhalation exposure risk assessments, a MOE of 300 is required. An extra factor of 3X should be applied to the conventional uncertainty factor of 100X to account for the lack of histopathology in the 7-day range-finding inhalation study in rats..

For intermediate-term and long-term inhalation exposure risk assessments, a MOE of 1000 is required. An extra factor of 10X should be applied to the conventional uncertainty factor of 100X to account for the lack of histopathology in the 7-day range-finding inhalation study in rats and the use of a short-term (7 days) study to evaluate intermediate-term and long-term inhalation exposure.

4. Recommendation for Aggregate Exposure Risk Assessments

There are no proposed residential home owner uses or other uses that will result in post-application residential exposure. Therefore, aggregate exposure risk assessment should be limited to Food + Water only.

3 CLASSIFICATION OF CARCINOGENIC POTENTIAL

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

§ 870.4300

MRID No.: 42248620, 44807223, 45150201

Discussion of Tumor Data: In a combined chronic toxicity/oncogenicity study (MRID 42248620 and MRID 44807223), 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) for up to 104 weeks. Groups of 10 rats of each sex per dose group were sacrificed at 52 weeks for interim evaluations.

A slightly increased incidence of thyroid gland follicular cell adenomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 100 ppm and 1000 ppm. The incidence was 8%, 6%, 10%, 14% and 14% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 13% and the mean was 6.5%. Also, a slightly increased incidence of thyroid gland follicular cell adenocarcinomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 1000 ppm. The incidence was 0%, 0%, 0%, 3% and 6% for the 0, 1, 10, 100 and 1000 ppm groups, respectively. The range of the historical control data (20 comparable studies at the same testing laboratory) was 0 to 5% and the mean was 1.4%. In addition, slightly increased incidences of pancreatic islet cell adenomas were also observed in the treated male rats, but these incidences were not dose-related and did not exceed the upper range of historical control data for the same tumor type (20 comparable studies at the same testing laboratory). Further, this type of tumor in this strain of rats is a common spontaneously occurring neoplasm. Therefore, the increased incidence of pancreatic islet cell adenomas observed in the treated male rats in this study was considered not likely to be related to treatment with the test material. High incidences of pituitary gland adenomas in males and females and of mammary gland fibroadenomas in females were also observed in all groups, including controls. These neoplasms are 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.

Adequacy of the Dose Levels Tested: The animals were adequately dosed as evidenced by decreased body weight gain at the 1000-ppm dose and microscopic lesions at the 100- and 1000-ppm doses. Males receiving the 1000-ppm diet weighed 6–16% ($p < 0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed $\leq 8\%$ less food than controls at each weekly interval. Females receiving the 1000-ppm diet weighed 7–24% ($p < 0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed $\leq 18\%$ less food than controls at each weekly interval. The food utilization factor or the food efficiency suggested that reduced body weight gain was due in part to toxicity of the test material.

Treatment related microscopic findings showed that the test material was toxic to 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. Liver toxicity was manifested by increased organ weight and increased incidences of gross and microscopic lesions. Absolute liver weights were increased by at least 7-27% and relative weights by 17-63% in both sexes at 1000 ppm. Gross lesions in the liver consisted of pale, swollen or accentuated markings on the livers at 1000 ppm in males and enlarged, pitted, or mottled livers at 1000 ppm in females. Treatment-related microscopic liver lesions at 100 ppm consisted of eosinophilic hepatocytes in 22% of females (8% in controls), centrilobular hepatocyte rarefaction and vacuolation in 8% of each sex (0% for controls), centrilobular sinusoidal dilatation in 10% of males and 18% of females (0% for male and 2% for female control), and pericholangitis in 18% of males and 14% of females (4% for male and 2% for female controls). Additional treatment-related liver lesions at 1000 ppm in main study group consisted of centrilobular hepatocyte vacuolation in males, centrilobular hepatocyte necrosis in females, and centrilobular fat and bile duct hyperplasia in both sexes.

Centrilobular hepatocyte vacuolation and centrilobular fat was also seen in 1000-ppm group male and female rats at interim sacrifice.

The incidences of exocrine atrophy of the pancreas in both sexes and acinar epithelial vacuolation or fat accumulation in females were increased at 1000; the incidence of exocrine atrophy was also increased at 100 ppm in females compared with that of control rats. The incidence of exocrine degranulation was increased in 1000-ppm group female rats at interim sacrifice but not in the main study. An increased incidence of thyroid follicular hyperplasia was observed in males at 1000 ppm (8% vs 2% for controls) and in females at 1000 ppm (10% vs 2% in controls). This finding may possibly be related to treatment with the test material. In male rats, the incidence of cortical tubular basophilia in the kidney was increased at 1000 ppm compared with that of the controls. Other treatment-related lesions included pneumonitis, alveolar adenomatosis, and alveolar epithelialization in 1000-ppm group males, alveolar epithelialization and alveolar macrophage aggregates in 1000-ppm group females, testicular atrophy in 100- and 1000-ppm group males, and spermatocoele granuloma also in 1000-ppm males. The incidence of sinus histiocytosis in the lymph nodes was increased in 1000-ppm group females. Histopathologic assessment of the brain and spinal cord of rats in the control and 1000-ppm dose groups showed no treatment-related effect on vacuolation of white matter.

2. Carcinogenicity Studies in Mice

§ 870.4200

Study #1:

MRID No.: 42208405, 44807220, 44807212

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

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

12% to 38%. The incidence for hepatocellular adenomas for high-dose males in this study (33%) slightly exceeded the upper range of historic controls (27%).

Adequacy of the Dose Levels Tested: The dosing was adequate for an oncogenicity study based on the liver and brain toxicity at 1000 ppm. The group mean liver weights adjusted for body weight were increased in males and females by 45% and 30%, respectively, at 1000 ppm compared to the controls, and by 15% in females at 100 ppm after 104 weeks of treatment ($p < 0.01$). Microscopic examination showed increased incidences of liver areas containing basophilic hepatocytes (controls, 12%; 1000 ppm, 38%, $p < 0.01$) and/or eosinophilic vacuolated hepatocytes (controls, 1%; 100 ppm, 8%, $p < 0.05$; 1000 ppm 19%, $p < 0.01$) in treated males compared to the controls. Increased incidences of granulomatous hepatitis of minimal severity were seen in high-dose males (controls, 11%; 1000 ppm, 25%, $p < 0.01$) and females (controls, 11%; 1000 ppm, 21%, $p < 0.01$). Higher incidences of aggregates of brown pigmented macrophages were seen in the livers of treated males (controls, 13%; 100 ppm, 27%, $p < 0.05$; 1000 ppm, 62%, $p < 0.01$) and females (controls, 15%; 1 ppm, 40%, $p < 0.01$; 10 ppm, 21%, NS; 100 ppm, 38%; 1000 ppm, 50%, $p < 0.01$). Granulomatous hepatitis and brown pigmented macrophage aggregates were most commonly seen in mice that survived until the end of the study.

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

An additional carcinogenicity study in mice (MRID 44807222, 44807221, 44807211) was performed subsequent to this study with higher dose levels of fluazinam (see below).

Study #2:

MRID No.: 44807222, 44807221, 44807211, 45201301

Discussion of Tumor Data: 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.

The following incidences of hepatocellular tumors and historical control data were taken from a Pathology Working Group (PWG) report dated August 24, 2000 (MRID 45201301), which was submitted after the original study report which was dated December 19, 1996.

Increased incidences of hepatocellular adenomas and of combined hepatocellular adenomas/carcinomas were observed in the treated male mice in this study. The percentage incidences of hepatocellular adenomas were 14%, 26%, 38% and 26% 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%, 8% (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 16%, 30%, 46% and 34% 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. No increases in hepatocellular tumor incidences were seen in the treated females compared to the controls. Historical control data provided in the PWG report (12 studies of 80 to 96 weeks duration at the same testing laboratory) showed the hepatocellular adenoma incidence in males ranged from 8% to 34% and the hepatocellular carcinoma incidence ranged from 2% to 16%. Historical control data was not provided for combined hepatocellular adenomas/carcinomas. The percentage incidence for hepatocellular adenomas for the 3000 ppm males in this study (38%) slightly exceeded the upper range of historic controls (34%).

Adequacy of the Dose Levels Tested: 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 an oncogenicity study based on increased mortality in females, decreased body weights in males and females, and liver and brain toxicity in males and females at 7000 ppm. 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, 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 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 "mod-

erate" 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.

3.3 Classification of Carcinogenic Potential

Combined results from two carcinogenicity studies in mice indicated that treatment of Crl:CD®-1 mice for up to 104 weeks resulted in statistically significant increases in the incidence of hepatocellular adenoma in male mice at 1000 and 3000 ppm. These increased incidences slightly exceeded the upper range of the historic control incidences. The hepatocellular carcinoma incidence was also increased in male mice at these same dose levels, but was not statistically significant and was within the range of historic control incidences. The incidences of combined hepatocellular adenoma and/or carcinoma were also increased in male mice at 1000 ppm, 3000 ppm ($p < 0.01$) and 7000 ppm ($p < 0.05$). **The increased incidence of hepatocellular adenomas and of combined hepatocellular adenomas and/or carcinomas observed in male mice at 1000, 3000 and 7000 ppm are considered to be treatment-related.** No significant increases in liver tumors were seen in treated female mice compared to controls.

The HIARC recommends that the results of these studies and all other pertinent data pertaining to the classification of carcinogenic potential of fluazinam be submitted to the HED Carcinogenicity Assessment Review Committee for review and evaluation.

4 MUTAGENICITY

There are 5 available mutagenicity studies on technical grade fluazinam. Results in all 5 studies were negative for mutagenic potential. One study (differential killing/growth inhibition assay in bacteria, MRID 42270607) was unacceptable because only one plate per dose, rather than 2 plates per dose, were used. The other 4 studies were acceptable.

There is a data gap for an *in vitro* forward gene mutation study in mammalian cells according to the post 1991 mutagenicity study requirements. An acceptable study of this type has not yet been submitted to the Agency. To satisfy this requirement, the applicant has already been asked to perform and submit either of the 2 following studies: mouse lymphoma assay, or Chinese Hamster Ovary (CHO) hgp_rt assay. In response to the Agency's request, the applicant has informed the Agency that it intends to perform and submit a new mouse lymphoma study by November, 2000.

Update: The applicant has recently submitted 2 additional mutagenicity studies which are presently under review. Preliminary evaluation of the studies indicated the following:

1) Autoradiographic DNA Repair Test on Rat Hepatocytes for CGA-143268 (also known as Fluazinam); 1984; MRID 45156901; this is not the type of study that would satisfy the data gap; study has several serious deficiencies and will be classified as an unacceptable study; study was negative for mutagenic potential.

2) L5178Y/TK +/- Mouse Lymphoma Mutagenicity Test; CGA-143268 (also known as Fluazinam); 1986; MRID 45156902; this is the type of study that would satisfy the data gap; but the study apparently was not done in replicate and therefore will be classified as an unacceptable study; study was negative for mutagenic potential.

Study #1

Executive Summary: In a reverse gene mutation assay in bacteria (MRID 42270605), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(*uvrA*) of *E. coli* were exposed to Fluazinam technical (Lot No. 8412-20, 95.3% a.i.) in DMSO. In the absence of mammalian metabolic activation (S9-mix), the TA-strains were exposed to Fluazinam concentrations of 0.0625, 0.125, 0.25, 0.5, 1.0 and 2.0 $\mu\text{g}/\text{plate}$ and WP2(*uvrA*) was exposed to concentrations of 15.6, 31.3, 62.5, 125 and 250 $\mu\text{g}/\text{plate}$. In the presence of S9-mix, the TA-strains were exposed to Fluazinam concentrations of 3.13, 6.25, 12.5, 25, 50 and 100 $\mu\text{g}/\text{plate}$ and WP2(*uvrA*) was exposed to concentrations of 31.3, 62.5, 125, 250 and 500 $\mu\text{g}/\text{plate}$. All platings were in duplicate. The S9-fraction was obtained from phenobarbital + 5,6-benzofurabon induced male Sprague-Dawley rat liver.

Fluazinam technical was tested up to cytotoxic concentrations. In the absence of S9-mix, complete growth inhibition (0 revertants/plate) was seen at 2 $\mu\text{g}/\text{plate}$ in all TA strains and also at 1 $\mu\text{g}/\text{plate}$ in TA1535 and TA1537. Less severe growth inhibition was seen at 1 $\mu\text{g}/\text{plate}$ in TA100 (mean of 17 revertants/plate compared to a mean of 96 revertants/plate in the solvent control). In WP2(*uvrA*) the number of revertants per plate was reduced at 250 $\mu\text{g}/\text{plate}$ to 4 revertants/plate compared to 30 revertants per plate for the solvent control. In the presence of S9-mix, complete growth inhibition (0 revertants/plate) was seen in all four TA-strains at 100 $\mu\text{g}/\text{plate}$ and in TA98, TA1535 and 1537 at 50 $\mu\text{g}/\text{plate}$. The mean number of revertants per plate in TA100 at 50 $\mu\text{g}/\text{plate}$ was 23 compared to the solvent control value of 120. In WP2(*uvrA*) the number of revertants per plate at 500 $\mu\text{g}/\text{plate}$ was reduced to 13 revertants/plate compared to 44 revertants/plate for the solvent control. No significant increase in the mutant frequency was seen. The number of revertants/plate was similar to the solvent control value at all Fluazinam concentrations, with or without S9-mix, in all tester strains. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5265 (§84-2)] for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

Study #2

Executive Summary: In a reverse gene mutation assay in bacteria (MRID 42270604), strains TA98, TA100, TA1535 and TA1537 of *S. typhimurium* and strain WP2(*uvrA*) of *E. coli* were exposed to Fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO. In the absence of mammalian metabolic activation (S9-mix), the TA-strains were exposed to Fluazinam concentrations of 0.0313, 0.0625, 0.125, 0.25, 0.5 and 1.0 $\mu\text{g}/\text{plate}$ and WP2(*uvrA*) was exposed to concentrations of 15.6, 31.3, 62.5, 125 and 250 $\mu\text{g}/\text{plate}$. In the presence of S9-mix, the TA-strains were exposed to Fluazinam concentrations of 31.3, 6.25, 12.5, 25, 50 and 100 $\mu\text{g}/\text{plate}$ and WP2(*uvrA*) was exposed to concentrations of 31.3, 62.5, 125, 250 and 500 $\mu\text{g}/\text{plate}$. All platings were in duplicate. The S9-fraction was obtained from phenobarbital + 5,6-benzofurabon induced male Sprague-Dawley rat liver.

Fluazinam technical was tested up to cytotoxic concentrations. In the absence of S9-mix, growth inhibition was seen at 1 $\mu\text{g}/\text{plate}$ in TA100 (0 revertants/plate), TA1535 (0 revertants/plate) and TA98 (8 revertants/plate compared to 24 revertants/plate in solvent control). In the presence of S9-mix, growth inhibition (0 revertants/plate) was seen in all four TA-strains at 50 $\mu\text{g}/\text{plate}$ and in the three TA-strains evaluated at 100 $\mu\text{g}/\text{plate}$. The number of revertants/plate was similar to the solvent control value at all Fluazinam concentrations, with or without S9-mix, in all tester strains. The solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

This study is classified as **Acceptable/guideline**. It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity (bacterial reverse gene mutation) data.

Study #3

Executive Summary: In a differential killing/growth inhibition assay in bacteria (MRID 42270607), strains H17 (*rec+*) and M45 (*rec-*) of *B. subtilis* were exposed to Fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO on paper disks at concentrations of 0.003, 0.01, 0.03, 0.1, and 0.3 $\mu\text{g}/\text{disk}$ in the absence of metabolic activation (S9-mix) and at concentrations of 0.3, 1, 3, 10, and 30 $\mu\text{g}/\text{disk}$ in the presence of S9-mix. The S9-fraction (purchased from Kikkoman Kenkyusho) was obtained from phenobarbital + 5,6-benzofurabon induced male Sprague-Dawley rat liver.

Fluazinam technical was tested up to cytotoxic concentrations. A preliminary cytotoxicity assay showed that the growth of both bacterial strains was inhibited at concentrations of 4 $\mu\text{g}/\text{disk}$ and higher with S9-mix and at concentrations of 0.032 $\mu\text{g}/\text{disk}$ and higher without S9-mix. In the differential killing assay, the growth of both bacterial strains was equally inhibited at Fluazinam

technical concentrations of 0.03 $\mu\text{g}/\text{disk}$ and above without S9-mix and at concentrations of 1 $\mu\text{g}/\text{disk}$ and above in the presence of S9-mix. There was no significantly greater growth inhibition or killing of the repair deficient M45 strain over that of the repair proficient H17 strain at any test material concentration, with or without S9-mix. In the absence of S9-mix, the diameter of the zone of inhibition around the disk at the maximum test material concentration was 8.7 mm with M45 and 7.4 mm with H17. Comparable values in the presence of S9-mix were 2.6 mm with M45 and 2.5 mm with H17. The negative, solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of greater growth inhibition or cell killing in repair-defective strains compared to repair competent strains.**

This study is classified as **Unacceptable/Guideline**. It does not satisfy the requirement for FIFRA Test Guideline [OPPTS 870.5500 (§84)]-2 for *in vitro* mutagenicity [bacterial DNA damage/repair] data because only one plate per dose was used in the differential killing assay and the guidelines require two or more plates per dose for a plate diffusion assay.

Study #4

Executive Summary: In a mammalian cell cytogenetics assay (chromosomal aberrations) (MRID 42270606), Chinese hamster lung fibroblast (CHL) cell cultures were exposed to Fluazinam technical (Lot No. 109, 95.3% a.i.) in DMSO, at concentrations of 1, 2 and 4 $\mu\text{g}/\text{mL}$ in the absence of metabolic activation (S9-mix) and at concentrations of 2.375, 4.75 and 9.5 $\mu\text{g}/\text{mL}$ in the presence of S9-mix. Cells were harvested at 24 and 48 hours after start of treatment in nonactivated studies and at 24 hours after start of treatment in activated studies. The S9-fraction was obtained from phenobarbital and 5,6-benzoflavone induced male Sprague-Dawley rat liver.

Fluazinam technical was tested up to cytotoxic concentrations. In a preliminary cytotoxicity assay, the IC_{50} in CHL cells was determined to be approximately 3.8 $\mu\text{g}/\text{mL}$ and 3.0 $\mu\text{g}/\text{mL}$ at 24 and 48 hours, respectively, in the absence of S9-mix and about 8.0 $\mu\text{g}/\text{mL}$ in the presence of S9-mix. Cells were harvested at 24 and 48 hours in the nonactivated study. Treatment times were 24 and 48 hours in the nonactivated assay and 6 hours in the activated assay. Two-hundred cells (100 per culture) per dose were evaluated. In the absence of S9-mix, the percentage of cells with structural aberrations (excluding gaps) at the 24 hour harvest time was 0.5%, 0.5% and 1.0% at 1, 2 and 4 $\mu\text{g}/\text{mL}$, respectively, compared to the solvent control value of 0.0% and the positive control value of 58%. Comparable values at the 48 hour harvest time were 0.0%, 0.0% and 1.0% at 1, 2 and 4 $\mu\text{g}/\text{mL}$, respectively, compared to the solvent control value of 0.5% and the positive control value of 29%. No polyploidy was seen at either harvest time. In the presence of S9-mix, the percentage of cells with structural aberrations (excluding gaps) were 1.0%, 0.0% and 2.0% at 2.375, 4.75 and 9.5 $\mu\text{g}/\text{mL}$, respectively, compared to the solvent control value of 1.0% and the positive control value of 50.5%. No polyploidy was seen. As a control for the metabolic activation assay, cultures were exposed to the same test material doses and exposure time as used in the presence of S9-mix but in its absence. The percentage of cells with structural aberrations in this assay (excluding gaps) were 0.0%, 3.5% and 3.0% at 2.375, 4.75 and 9.5 $\mu\text{g}/\text{mL}$, respectively, compared to the solvent control value of 0.5% and the positive control value of 0.0% (expected for cyclophosphamide

which requires activation). Solvent and positive controls induced the appropriate response. **There was no evidence of chromosomal aberrations induced over background.**

This study is classified as **Acceptable/guideline**. It satisfies the requirement for FIFRA Test Guideline [OPPTS 870.5375 (§84)]-2 for in vitro cytogenetic mutagenicity data.

Study #5

Executive Summary: In an ICR (Crj:CD-1) mouse bone marrow micronucleus assay (MRID 44807224), five mice/sex/dose were treated once via oral gavage with IKF-1216 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.

IKF-1216 technical was tested to a limit concentration of 2000 mg/kg. The maximum tolerated dose was determined to be 3000 mg/kg in a preliminary toxicity assay (3/3 male mice died at 4000 mg/kg and 1/3 died at 5000 mg/kg). There were few signs of toxicity during the micronucleus studies. No deaths occurred in either the first or second micronucleus study. The only clinical signs seen in the first study were decreased spontaneous motor activity and piloerection at 2000 mg/kg in one IKF-1216 treated male. In the second study, two males each from the 500, 1000 and 2000 mg/kg dose groups showed loose stools at 5 hours post-treatment and one male from the 2000 mg/kg dose group showed loose stools at 24 hours post-treatment. One female from the 1000 mg/kg group showed loose stools at 5 hours post-treatment and one male each from the 1000 and 2000 mg/kg groups showed soiled fur around the genital region at 24 hours post-treatment. No adverse clinical signs were seen in the solvent or positive controls in either study. There was no statistically significant increase in the frequency of micronucleated PCEs over solvent control values at any IKF-1216 technical concentration or harvest time in either sex. The solvent and positive controls induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow at any IKF-1216 concentration or treatment time used in this study.**

This study is classified as **Acceptable/Guideline**. It satisfies the requirement for FIFRA Test Guideline OPPTS 870.5395(§ 84-2) for *in vivo* cytogenetic mutagenicity data.

5 FOPA CONSIDERATIONS

5.1 Adequacy of the Data Base

The available toxicology data base for fluazinam includes the following acceptable studies:

1. Acute neurotoxicity study, rats § 870.6200, MRID 44807210

- 2. Subchronic neurotoxicity study, rats § 870.6200, MRID 44807217
- 3. Developmental toxicity study, rats § 870.3700, MRID 42248613
- 4. Developmental toxicity study, rabbits § 870.3700, MRID 42248616
- 5. 2-Generation reproduction study, rats § 870.3800, MRID 42248619

The available toxicology data base for fluazinanam is adequate for assessment of potential hazard to infants and children.

5.2 Neurotoxicity Data

5.2.1 Acute neurotoxicity study, rats § 870.6200, MRID 44807210

Executive Summary: See 2.1.2 General Population (including infants and children)

5.2.2 Subchronic neurotoxicity study, rats § 870.6200, MRID 44807217

Executive Summary: In two subchronic oral neurotoxicity studies (MRID 44807217 & MRID 44807218), groups of 10 male and 10 female Crl:CD BR rats were fed diets containing 0, 300, or 1000 ppm fluazinanam (MRID 44807217, 96.9%, Lot No. 6109) or 0, 1000, 2000, or 3000 ppm fluazinanam (MRID 44807218, 98.4%, Lot No. 9601-2) for 13 weeks. Achieved doses were 20.7, 69-74, 149, and 233 mg/kg/day for males in the 300, 1000, 2000, and 3000 ppm groups, respectively; and 23.4, 81-89, 175, and 280 mg/kg/day for females in the 300, 1000, 2000, and 3000 ppm groups, respectively. Functional Observational Battery (FOB) and Motor Activity (MA) assessments were performed prior to treatment and during weeks 4, 8, and 13 of treatment. Body weights, food consumption, and clinical signs were monitored throughout the study. At the end of the treatment period, all rats were perfused *in situ*. The brain from all rats was removed, weighed, and measured and 5 males and 5 females from the control and high-dose groups of each study were subjected to neuropathological evaluation.

There were no treatment-related deaths or clinical signs. At the end of the study, group mean body weight gains were significantly ($p < 0.01$) decreased in females in and above the 1000 ppm groups and in males in the 2000 and 3000 ppm groups. Similarly, cumulative food consumption was decreased in males ($p < 0.01$) and females ($p < 0.05$) fed 2000 and 3000 ppm fluazinanam. Food efficiency was decreased in males at 3000 ppm and a dose-related decrease in food efficiency was observed in females in all treatment groups.

No treatment-related FOB or MA effects were observed. Brain weights of females in the 3000 ppm group were 8% lower ($p < 0.01$) than controls; however, no supporting pathology was observed. No treatment-related gross effects or histopathology were observed.

Under the conditions of these studies, the neurotoxicity NOAEL is 3000 ppm for male and female rats (233 mg/kg/day for males and 280 mg/kg/day for females). A neurotoxicity LOAEL was not identified.

Comments on acute and subchronic neurotoxicity studies in rats: The soft stools observed in males and females on the day of dosing and the decreased motor activity values observed in females on the day of dosing in the acute neurotoxicity study on rats at ≥ 1000 mg/kg are considered to be manifestations of acute general systemic toxicity and not a direct neurotoxic response to the administration of the test material. See the Executive Summary for this study in 2.1.2 General Population (including infants and children) for a more detailed explanation of this conclusion. No significant signs of neurotoxicity were observed in either the acute or subchronic neurotoxicity studies on rats.

5.2.3 Evidence of neurotoxicity from other oral toxicity studies on fluazinam

A neurotoxic lesion described as vacuolation of the white matter of the central nervous system (CNS) was observed initially in long-term (1-2 year) guideline chronic studies on mice and dogs and later, upon careful re-examination of the CNS, also in shorter-term (4-week to 90-day) guideline subchronic studies on mice and dogs. This lesion was observed during the (light) microscopic examination of several tissues of the CNS, occurring most frequently in brain (sections of cerebrum and/or sections of cerebellum, pons, medulla, midbrain) and less frequently in cervical spinal cord. Although this lesion was also observed in control animals, the increased incidence and/or severity of the lesion in test animals was clearly treatment-related and dose-related.

It is noteworthy that the lesion was not observed in any guideline studies on rats, even though a careful re-evaluation of the CNS was performed for all critical studies, including the major chronic (MRID 42248620) and subchronic (MRID 42248610) studies. Further investigation of this lesion in a series of 8 additional special studies (see below), however, demonstrated the lesion could also be induced in rats, albeit at higher dose levels than used in the guideline studies.

In guideline studies, the lesion was observed in the following studies:

1. 2-year carcinogenicity study in mice (1996) MRID 44807222, 44807221,
44807221

Dose levels tested:

0, 1000, 3000 & 7000 ppm

M: 0, 126, 377 & 964 mg/kg/day

F: 0, 162, 453 & 1185 mg/kg/day

An increased incidence and severity of vacuolation of white matter of the brain was observed in males at 126, 377 and 964 mg/kg/day and in females at 162, 453 and 1185

mg/kg/day. An increased incidence and severity of vacuolation of white matter of the cervical spinal cord was observed in males at 377 and 964 mg/kg/day.

2. 2-year carcinogenicity study in mice (1988) MRID 42208405, 44807220,
44807212

Dose levels tested:

0, 0, 1, 10, 100 & 1000 ppm

M: 0, 0, 0.1, 1.1, 10.7 & 107 mg/kg/day

F: 0, 0, 0.1, 1.2, 11.7 & 117 mg/kg/day

An increased incidence and/or severity of vacuolation of white matter of the brain was observed in males at 107 mg/kg/day and in females at 117 mg/kg/day. An increased incidence and/or severity of vacuolation of white matter of the cervical spinal cord was not observed in males or in females in this study.

3. 4-Week range-finding feeding study in mice (1994) MRID 44807211

Dose levels tested:

0, 3000, 5000 & 7000 ppm

M: 0, 555, 938 & 1200 mg/kg/day

F: 0, 658, 1050 & 1400 mg/kg/day

An increased incidence and severity of vacuolation of white matter of the brain was observed in males at 555, 938 and 1200 mg/kg/day and in females at 1400 mg/kg/day. An increased incidence and severity of vacuolation of white matter of the cervical spinal cord was observed in males at 938 and 1200 mg/kg/day.

4. 1-Year chronic oral study in dogs, capsules (1987) MRID 42270603, 44807219

Dose levels tested:

M: 0, 1, 10 & 50 mg/kg/day

F: 0, 1, 10 & 50 mg/kg/day

An increased incidence and severity of vacuolation of white matter of the brain was observed in males at 50 mg/kg/day and in females at 50 mg/kg/day. Vacuolation of white matter of the cervical spinal cord was also observed in females at 50 mg/kg/day.

5. 90-day subchronic oral study in dogs, capsules (1984) MRID 42248611, 44807215

Dose levels tested:

M: 0, 1, 10 & 100 mg/kg/day

F: 0, 1, 10 & 100 mg/kg/day

A marginally (equivocal) increased incidence of vacuolation of white matter of the brain (cerebrum) was observed in males at 100 mg/kg/day and in females at 100 mg/kg/day. The spinal cord was not re-examined in this study.

Special Mechanistic Studies

A series of 8 special mechanistic studies was designed to determine, if possible, the etiology of the vacuolation of the white matter of the CNS observed in the guideline studies on fluazinan and to further evaluate several additional characteristics of the lesion as follows:

1. Determination of specific chemical(s) responsible for inducing the lesion in mice, dogs and rats.

Fluazinan, per se, was not responsible for the induction of this lesion. An analysis of the effects of nine impurities present in Fluazinan Technical revealed that one single impurity, Impurity-5, is solely responsible for the appearance of white matter vacuolation. Impurity-5 was present in the various lots of Fluazinan Technical used for toxicity testing at concentrations ranging from <0.005% to 0.2% w/w ⁽¹⁾. With respect to the ability of Impurity-5 to produce white matter vacuolation, there seems to be a non-linear dose-response with a clear threshold below which no effect occurs.

⁽¹⁾ The maximum level of Impurity-5 permitted in the specification for technical grade fluazinan is currently 0.3% w/w, but may be reduced to 0.1% w/w. See letter from Gary L. Eilrich, Vice President, Regulatory Affairs, ISK Biosciences Corporation to Mr. Jim Jones, Director, RD, OPP, dated August 3, 2000).

Single oral (gavage) doses of 5 mg/kg of Impurity-5 (99.5% purity) given to fasted mice caused course fur, staggering gait, sedation for 20 hours and decreased body weight. All mice were sacrificed *in extremis* at 24 hours. Increased brain weights, edema of the brain and vacuolation of the brain were observed in the treated animals.

In rats, single oral (gavage) doses of 5000 mg/kg of the analytical standard of fluazinan (containing <0.0005% Impurity-5) caused no vacuolation of the white matter of the brain whereas similar doses of Fluazinan Technical did cause vacuolation of the white matter of the brain.

2. Determination of dose-response relationships for Fluazinan Technical

In the mouse and rat, vacuolation of the white matter in the brains (and optic nerves of mice) was observed only when high doses of Fluazinan Technical were administered. In mice, single oral (gavage) doses of 3000 mg/kg of Fluazinan Technical (95.3% purity) caused decreased locomotor activity, prone position, paralysis of hind legs, tremors, staggering gait and moribundity. Edema of the brain and vacuolation of the white matter of

the brain were observed in the treated animals. Assuming a concentration of 0.1 % Impurity-5 in Fluazinam Technical, this dose (3000 mg/kg) is approximately equal to a dose of 3 mg/kg of Impurity-5.

In mice, administration of Fluazinam Technical (containing 0.12% Impurity-5) in the diet for 4 days at 20,000 ppm resulted in abnormal behavior on day 4 and trace edema of the brain. In mice, administration of Fluazinam Technical (containing 0.12% Impurity-5) in the diet for 4 days at 7,000 ppm resulted in trace edema of the brain. In mice, administration of Fluazinam Technical (containing 0.12% Impurity-5) in the diet for 28 days at 7,000 ppm resulted in vacuolation of the white matter of the brain. Vacuolation of white matter was observed in the brains of all treated animals sacrificed at the end of treatment.

In rats, single oral (gavage) doses of 5000 mg/kg of Fluazinam Technical (containing 0.12% to 0.20% Impurity-5) caused decreased locomotor activity, prone position, paralysis of hind legs, tremors, staggering gait and moribundity. Edema of the brain and vacuolation of the white matter of the brain were observed in the treated animals. Assuming a concentration of 0.12 % Impurity-5 in Fluazinam Technical, this dose (5000 mg/kg) is approximately equal to a dose of 6 mg/kg of Impurity-5.

In rats, administration of Fluazinam Technical (containing 0.12% Impurity-5) in the diet for 14 days at 30,000 ppm (1742 mg/kg/day) and at 10,000 ppm (714 mg/kg/day) resulted in edema of the brain and minimal to moderate vacuolation of the white matter of the brain at 30,000 ppm, and trace vacuolation of the white matter of the brain at 10,000 ppm.

3. Reversibility of the CNS lesion

White matter vacuolation in the CNS was fully reversible, and no progression of this abnormality was observed with time. In the 14-day dietary study in rats described above, recovery from the CNS lesion was also studied. Some rats were allowed to recover for an additional 25 days (no fluazinam in the diet) and then examined. For 30,000 ppm animals, only trace vacuolation of the white matter of the brain was observed. For 10,000 ppm animals, no vacuolation of the white matter of the brain was observed.

In the mouse study described above in which Fluazinam Technical was administered in the diet for 4 days at 20,000 ppm, or for 4 days at 7,000 ppm, or for 28 days at 7,000 ppm, vacuolation of white matter was observed in the brains of all treated animals sacrificed at the end of treatment. This abnormality was not observed after a 24-day recovery period among animals treated at 7,000 ppm for 4 days or after 56 days among those treated at 7,000 ppm for 28 days or at 20,000 ppm for 4 days.

4. Differences in species and sex susceptibility

In a series of studies, no significant differences in susceptibility or in incidence or severity of vacuolation of the white matter of the CNS were observed between species (mice, dogs, or rats). Similarly, no significant differences were attributed to sex.

5. Differences in age-related susceptibility

An age-related increased sensitivity was identified in mice and rats at 10 weeks compared to 3 weeks of age.

Impurity-5 was administered to groups of male mice aged 3, 10, or 24 weeks by a single oral gavage at 2.5 mg/kg. The severity of white matter vacuolation increased with age until about ten weeks, then plateaued at 24 weeks as observed under the limitations of this study.

The difference in age sensitivity was comparable between rats and mice as displayed in another oral gavage study using 3 and 10 week-old mice and rats dosed with 0 or 0.5 mg/kg of Impurity-5. Microscopic observation of the brains of treated animals revealed white matter vacuolation with slightly different severity between the respective age groups. The severity of these lesions was similar for both species of the same age, but greater in 10 week old animals as compared to 3 week old animals.

6. Electron Microscopy of the CNS lesion

Electron microscopy of the white matter (cerebellum) of mice treated with fluazinam technical indicated that treatment-related effects were confined to the myelin sheaths. Large vacuoles were observed in the intramyelin sheaths due to the accumulation of fluid between the sheaths. The nucleus and mitochondria in oligodendroglia were observed to remain intact, suggesting no damage to these cells. The myelin sheaths appeared to recover completely during a recovery period of up to 56 days.

Comments on Threshold Dose for Vacuolation of the White Matter of the CNS

There appears to be a non-linear dose-response with a clear threshold below which no effect occurs. When the guideline data were analyzed and presented graphically [see Figure 1 (page 100) in the Overview Document (MRID 44807207) prepared by the applicant], it was apparent that no white matter vacuolation occurred when the dose of Impurity-5 was below about 0.1 mg/kg/day. The lowest effect level for white matter vacuolation was observed in the dog chronic study at 0.1 mg/kg/day of Impurity-5 (equivalent in this study to 50 mg/kg/day of fluazinam technical containing 0.2% of Impurity-5).

Based on a consideration of all the available data and information relating to this treatment-related neurotoxic lesion, the HIARC concluded that a LOAEL of 0.1 mg/kg/day and a NOAEL of 0.02 mg/kg/day for CNS effects could be established for Impurity-5.

NOAEL (for CNS effects) = 0.02 mg/kg/day of Impurity-5

At the current maximum concentration of Impurity-5 in technical grade fluazinam of 0.3% w/w (from specifications in most recent CSF), this is equivalent to:

NOAEL (for CNS effects) = 6.67 mg/kg/day of technical grade fluazinam

$$\text{Calculation: } \frac{0.02 \text{ mg/kg/day}}{0.3\%} = \frac{x \text{ mg/kg/day}}{100\%} \quad x = 6.67$$

This is to be compared to:

NOAEL (for chronic effects for chronic RfD) = 1.1 mg/kg/day of technical grade fluazinam

The chronic RfD of 0.011 mg/kg/day for the general population (including infants and children) determined in 2.2 Chronic Reference Dose (RfD) is therefore protective of the CNS effects caused by Impurity-5 present in technical grade fluazinam at levels up to 0.3% w/w.

Other pertinent neurotoxicity data from the literature: None available.

5.3 Developmental Toxicity

5.3.1 Range-finding developmental toxicity study in rats [§ 870.3700, MRID 42248612]

Executive Summary: In a developmental range-finding toxicity study (MRID 42248612), 7 pregnant CD (Sprague-Dawley origin) rats per group were administered B-1216 (98.5%; Lot No. 8303-2) by gavage in corn oil at doses of 0, 1, 10, 100, and 1000 mg/kg/day on gestation days (GD) 6-15, inclusive. On GD 20, dams were sacrificed and necropsied, and the number and location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea were recorded, as well as the weights of the ovaries, empty uteruses, and adrenal and pituitary glands. All fetuses were weighed, sexed and examined externally, and approximately half of each litter was processed for visceral examination, and the remaining half of each litter was examined by fresh dissection then processed for skeletal examination.

Maternal toxicity was evident at 1000 mg/kg/day. Two animals were found dead on GD 13 and the remaining 5 were sacrificed *in extremis* between GD 12 and 14. Prior to death the high-dose animals exhibited clinical signs of stained and ungroomed coats, lethargy, hunched posture, ataxia,

flaccid muscles, and salivation. Post mortem findings included decreased thymus size and gastrointestinal tract disturbances. Marked weight loss was observed at 1000 mg/kg/day after GD 7, and mean absolute body weights were 74-86% of those of controls during GD 10-13. Body weight and survival were not affected in the 1, 10, and 100 mg/kg/day groups.

There were no differences between the control group and the 1, 10, or 100 mg/kg/day groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, resorptions, or fetal sex ratios. At 100 mg/kg/day, mean fetal weight was marginally decreased as compared with concurrent controls but fell within the range of historical control data. The incidence of incomplete ossification of sternbrae was increased in the 100 mg/kg/day group as compared to concurrent and historical controls (38.9% of fetuses and 7/7 litters vs. 11.8% and 3/7 litters for concurrent controls and a historical control range of 1.1-28.3%); however, there was no evidence of delayed ossification in any other bone types. The incidence rate for litters containing fetuses with additional (14th) rib(s) was 1/7, 2/7, 2/7, and 3/7 for the 0, 1, 10, and 100 mg/kg/day groups, respectively, with the percentage of affected fetuses slightly increased in all treated groups as compared with concurrent and historical controls. Treatment with B-1216 did not result in an increased incidence of fetal malformations.

Therefore, it was concluded that an appropriate high dose for the main developmental toxicity study (MRID 42248613) would be greater than 100 mg/kg/day but less than 1000 mg/kg/day. The dose levels chosen were 0, 10, 50, and 250 mg/kg/day.

This study is classified as **Acceptable/Nonguideline** and fulfills its intent as a range finding study for a developmental toxicity study [870.3700 (§83-3a)] in rats.

Comment: The HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

5.3.2 Developmental toxicity study in rats **[§ 870.3700, MRID 42248613]**

Executive Summary: In a developmental toxicity study (MRID 42248613), 20 presumed pregnant Sprague-Dawley CD rats per group were administered Fluazinam (98.5% a.i, Lot No.: 8303-2) by gavage in corn oil at doses of 0, 10, 50 and 250 mg/kg/day on gestation days (GD) 6-15, inclusive. Controls were treated with corn oil (vehicle). On GD 20, all dams were sacrificed and examined. All fetuses were weighed, sexed and examined for external malformations and variations. Approximately half of the fetuses from each litter were examined for soft tissue effects and half were stained with Alizarin red S and examined for skeletal effects.

At 250 mg/kg/day, statistically significant reductions in body weight gain during treatment (30 gm vs 51 gm for controls on GD 6-15; $p < 0.01$; most pronounced during GD 6-8), statistically significant reductions in food consumption during treatment (13 mg/kg/day vs 17 mg/kg/day for controls on GD 6-8; $p < 0.01$), increased water consumption (during GD 6-11) and an increased

incidence of urogenital staining (most pronounced during GD 6-8) were considered to be treatment-related. **The maternal toxicity LOAEL is 250 mg/kg/day based on decreased body weight gain, decreased food consumption, increased water consumption, and increased urogenital staining during treatment. The maternal toxicity NOAEL is 50 mg/kg/day.**

At 250 mg/kg/day, statistically significant decreased mean fetal body weights (2.81 gm vs 3.19 gm for controls, $p < 0.001$, below historical control range), statistically significant decreased placental weights (0.47 gm vs 0.54 gm for controls, $p < 0.05$, within historical control range), increased fetal incidence of facial/palate clefts (10 fetuses in 3 litters vs none in controls), increased fetal incidence of diaphragmatic hernia (7 fetuses in 2 litters vs none in controls), delayed ossification in a number of bone types, greenish amniotic fluid (10.5% fetal incidence vs 0.0% in controls) and possible increased late resorption/postimplantation loss (0.55 late resorptions/dam vs 0.05 late resorptions /dam for controls, within historical control range; and 11.0% postimplantation loss vs 4.2% postimplantation loss for controls, within historical control range) were considered to be treatment-related. **The developmental toxicity LOAEL is 250 mg/kg/day based on decreased fetal body weights; decreased placental weights; increased fetal incidences of facial/palate clefts, diaphragmatic hernia and delayed ossification in several bone types; greenish amniotic fluid and possible increased late resorptions and postimplantation loss. The developmental toxicity NOAEL is 50 mg/kg/day.**

This developmental toxicity study in rats is classified **Acceptable/Guideline** and **satisfies** the Subdivision F guideline requirement for a developmental toxicity study in rats [OPPTS 870.3700 (OPP 83-3a)]. No major deficiencies were noted in this study.

Comment: Although quantitative evidence of increased susceptibility of fetuses to fluazinam was not observed in this study, the HIARC considered the increased fetal incidences of facial/palate clefts and other rare deformities observed in this study to be qualitative evidence of increased susceptibility of fetuses to fluazinam.

5.3.3 Developmental toxicity study in rabbits [§ 870.3700, MRID 42248616]

Executive Summary: See Executive Summary for Acute Reference Dose (RfD), Females 13-50 Years.

Comment: The HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

5.4 Reproductive Toxicity [§ 870.3800, MRID 42248619, 42208406, 42248618]

Executive Summary: Technical grade fluazinam (95.3 % a.i.) was administered to groups of 24 male and 24 female Sprague-Dawley rats at dietary concentrations of 0, 20, 100, or 500 ppm for

two generations (MRID 42248619, 42208406, 422248618). One litter was produced in each generation. Mean pre-mating doses were 1.5, 7.3, and 36.6 mg/kg/day, respectively for F₀ males and 1.7, 8.4, and 42.1 mg/kg/day, respectively for F₀ females. Mean pre-mating doses were 1.9, 9.7, and 47.3 mg/kg/day respectively, for F₁ males and 2.2, 10.6, and 53.6 mg/kg/day, respectively, for F₁ females. F₁ adults were chosen from the F₁ pups and weaned onto the same diet as their parents. Animals were given test or control diet for 11 weeks before mating within the same dose group. All animals were continuously exposed to test material either in the diet or during gestation and lactation until sacrifice.

There were no deaths or clinical signs of toxicity that were attributable to the presence of fluazinam in the diet. Mean body weight, body weight gain, food consumption and food efficiency among all groups of F₀ males and F₀ females treated with 20 or 100 ppm and F₀ males treated with 500 ppm were similar to the control group means. The F₀ females treated with 500 ppm of the test diet had significantly decreased (82% of control value, $p < 0.001$) overall body weight gain and food consumption (96% of control value, $p < 0.05$) for the pre-mating period. The F₁ males and females treated with 20 or 100 ppm had mean body weights, body weight gains, food consumption, and food efficiencies that were similar to their respective control group means. The F₁ animals treated with 500 ppm had significantly decreased mean body weight gain and food consumption values that were 88% and 92% ($p < 0.001$ and $p < 0.01$) and 85% and 93% ($p < 0.001$ and $p < 0.01$) of the control values for males and females, respectively for the pre-mating period. The decreased body weights continued into gestation for females treated with 500 ppm of both generations; some recovery was made during lactation. The relative liver weights of F₀ and F₁ males and F₀ females treated with 500 ppm were significantly increased compared to the control group. Histopathological findings included an increased incidence of peri-acinar hepatocytic fatty changes and a decreased incidence of hepatic glycogen pallor among F₀ males treated with 500 ppm compared to the control group. Males in the F₁ generation treated with 100 or 500 ppm also had significantly increased incidences of peri-acinar hepatocytic fatty changes compared to the control groups. **The NOAEL for parental toxicity is 20 ppm (1.9 mg/kg/day) and the LOAEL is 100 ppm (9.7 mg/kg/day), based on liver pathology (increased incidences of peri-acinar hepatocytic fatty changes) in F₁ males.**

The fertility index for males and females treated with 500 ppm of the test substance was slightly decreased (n.s.) for F₁ parents compared to the control group. The number of implantation sites observed in F₁ dams was decreased significantly ($p < 0.05$) at 500 ppm (12.2 vs 15.3 in controls) and marginally (n.s.) at 100 ppm (13.1 vs 15.3 in controls). Mean litter size on day 1 was slightly decreased (n.s.) in the 500 ppm groups compared to the control groups in both generations. Mean litter size on day 4 was slightly decreased (n.s.) in the 500 ppm group for F₁ litters, but was significantly decreased ($p < 0.05$) in the 500 ppm group for F₂ litters (9.8 ± 3.7 for 500 ppm vs 12.4 ± 3.0 for controls). Pup survival was similar between the treated and control groups for both generations. **The NOAEL for reproductive toxicity is 100 ppm (10.6 mg/kg/day) and the LOAEL is 500 ppm (53.6 mg/kg/day), based on a decreased number of implantation sites and decreased litter sizes to day 4 post partum for F₁ females (F₂ litters).**

Mean overall body weight gain during lactation was significantly decreased (10-13%), among pups in the 500 ppm groups in both generations. The most pronounced effect on pup weight gains occurred between lactation days 7-21. Absolute body weights, however, were not significantly decreased compared to the control groups at any time point during lactation. A slightly decreased developmental time for pinna unfolding, hair growth and eye opening, particularly in the F₂ pups, was observed. **The NOAEL for developmental toxicity is 100 ppm (8.4 mg/kg/day) and the LOAEL is 500 ppm (42.1 mg/kg/day), based on decreased body weight gain during lactation for both F₁ and F₂ pups.**

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

Comment: The HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

5.5 Additional Information from Literature Sources

None available

5.6 Determination of Susceptibility

In the developmental toxicity study in rats (MRID 42248613), the HIARC considered the increased fetal incidences of facial/palate clefts and other rare deformities observed in this study to be qualitative evidence of increased susceptibility of fetuses to *in utero* exposure to fluazinam. Quantitative evidence of increased susceptibility of fetuses to fluazinam was not observed, however, in this study.

In the developmental toxicity study in rabbits (MRID 42248616), The HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to *in utero* exposure to fluazinam was observed in this study.

In the 2-generation reproduction study in rats (MRID 42248619, 42208406, 42248618), the HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

5.7 Recommendation for a Developmental Neurotoxicity Study

5.7.1 Evidence that does suggest requiring a Developmental Neurotoxicity study:

In a series of chronic and subchronic studies on fluazinam on rats, mice and dogs, a treatment-related neurotoxic lesion described as vacuolation of the white matter of the CNS (brain and/or

cervical spinal cord) was observed when high doses of Fluazinam Technical were administered to the animals. It was determined that this lesion was caused solely by an impurity in Fluazinam technical (Impurity-5) when this impurity was present in the test material such that test animals received doses of Impurity-5 of 0.1 mg/kg/day and higher. When this impurity was present in the test material such that test animals received doses of Impurity-5 of 0.02 mg/kg/day or lower, the neurotoxic lesion was not observed.

An age-related difference in sensitivity to vacuolation of the white matter of the CNS caused by Impurity-5 was also demonstrated in rats and mice. Increased sensitivity to this neurotoxic effect was observed in rats and mice at 10 weeks compared to 3 weeks of age.

In the developmental toxicity study in rats (MRID 42248613) the HIARC considered the increased fetal incidences of facial/palate clefts and other rare deformities observed in this study to be qualitative evidence of increased susceptibility of fetuses to *in utero* exposure to fluazinam. Quantitative evidence of increased susceptibility of fetuses to fluazinam was not observed in this same study.

In the range-finding developmental toxicity study in rats (MRID 42248612), at 1000 mg/kg/day (highest dose tested), 2 dams were found dead on GD 13 and the remaining 5 were sacrificed *in extremis* between GD 12 and 14. Prior to death the high-dose animals exhibited clinical signs of neurotoxicity, including stained and ungroomed coats, lethargy, hunched posture, ataxia, flaccid muscles, and salivation. These signs were not observed in the survivors at this dose level or at lower dose levels of test material in the study.

5.7.2 Evidence that do not support the need for a Developmental Neurotoxicity study

In the acute and subchronic neurotoxicity studies on rats (MRID 44807210 and 44807217/44807218, respectively), no toxicologically significant signs of neurotoxicity were observed. The highest dose of Impurity-5 in the subchronic neurotoxicity study, however, was only 0.03 mg/kg/day.

In the definitive developmental toxicity studies on rats and rabbits (MRID 42248613 and MRID 42248616, respectively), treatment-related increased incidences of malformations of nervous system tissues were not observed.

In the developmental toxicity study in rabbits (MRID 422486516), The HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to *in utero* exposure to fluazinam was observed in this study.

In the 2-generation reproduction study in rats (MRID 422486519, 42208406, 42248618), the HIARC determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

5.7.3 Based on a consideration of the evidence described above, the HIARC recommended that a developmental neurotoxicity study in rats be required in order to more clearly and fully characterize the toxicity of this chemical.

Developmental neurotoxicity study, rats

§ 870.6300

Test Material: to be technical grade fluazinam containing maximum level of Impurity-5 permitted in specification for technical grade fluazinam (currently 0.3%, but may be reduced to 0.1%. See letter from Gary L. Eilrich, Vice President, Regulatory Affairs, ISK Biosciences Corporation to Mr. Jim Jones, Director, RD, OPP, dated August 3, 2000).

Proposed Protocol: to include full neurohistopathological examination of dams. Protocol to be submitted to EPA for comment prior to commencement of study.

Based on a consideration of the results in the developmental neurotoxicity study in rats required above, HIARC will subsequently determine whether a repeat of the subchronic neurotoxicity study in rats (§ 870.6200) will also be required to support the registration of fluazinam.

Subchronic neurotoxicity study, rats

§ 870.6200

Requirement: reserved; to be determined at a later time (see above).

6 HAZARD CHARACTERIZATION

Technical grade fluazinam (lot #109, 95.3% purity) had an acute oral LD50 in rats of 4100-4500 mg/kg (Toxicity Category III), whereas two other lots of technical grade fluazinam (lot #8412-20, 95.3% purity and lot #1/87, 97.9% purity) had acute oral LD50s in rats of >5000 mg/kg (Toxicity Category IV). The acute dermal LD50 of technical grade fluazinam in rats was >2000 mg/kg (Toxicity Category III) and the acute inhalation LC50 in rats was 0.463-0.476 mg/L (Toxicity Category II). Technical grade fluazinam was extremely irritating in a primary eye irritation study in rabbits (Toxicity Category I) and slightly irritating in a primary skin irritation study in rabbits (Toxicity Category IV). In dermal sensitization studies in guinea pigs, technical grade fluazinam (96.7% purity) was positive, but ultra-purified fluazinam (100% purity) was negative for dermal sensitization.

In a battery of acute toxicity studies applicable to Omega 500F (EPA file symbol 71512-R, a flowable liquid concentrate of fluazinam containing 40% active ingredient), the acute oral LD50 in rats was >5000 mg/kg (Toxicity Category IV); the acute dermal LD50 in rabbits was >2000 mg/kg (Toxicity Category III) and the acute inhalation LC50 in rats was 3.0-3.4 mg/L (Toxicity Category IV). In a primary eye irritation study in rabbits, it was slightly irritating (Toxicity Category III); in

a primary skin irritation study in rabbits, it was moderately irritating (Toxicity Category II) and in a dermal sensitization study in guinea pigs, it was positive for dermal sensitization.

In subchronic and chronic oral, dermal and inhalation studies in rats, dogs and/or mice, the liver appeared to be the primary target organ. Signs of liver toxicity were consistently observed at or slightly above the LOAEL in all three species. These signs frequently included changes in clinical chemistries indicative of liver toxicity (e.g. increased serum alkaline phosphatase), increased absolute and/or relative liver weights, increased incidences of macroscopic liver lesions (e.g. pale, enlarged, pitted, mottled, accentuated markings) and increased incidences of a variety of microscopic liver lesions. Microscopic liver lesions included eosinophilic or basophilic hepatocytes, rarefied or vacuolated hepatocytes, altered hepatocytic foci, hepatocytic single cell necrosis, hepatocytic hypertrophy, hepatocellular fatty changes, increased brown pigmented macrophages, sinusoidal chronic inflammation, pericholangitis, and bile duct hyperplasia.

Treatment-related effects were also observed in other organs in subchronic and chronic oral, dermal and inhalation studies in rats, dogs and/or mice, but these effects were not regularly noted in all three species or in all studies in a given species. In rats, these effects included decreased body weight gain, decreased food consumption, mild anemia, increased serum cholesterol, increased serum phospholipid, increased serum aspartate aminotransferase, testicular atrophy, slightly increased testes weights (inhalation study), pancreatic exocrine atrophy, increased lung weights, increased alveolar adenomatosis, epithelialization and macrophages, thyroid follicular cell hyperplasia, and possibly increased thyroid follicular cell adenomas and adenocarcinomas in males (but not in females). In dogs, these effects included increased salivation, increased nasal dryness, grey mottling of the retina, mild anemia, increased serum alkaline phosphatase and gastric lymphoid hyperplasia. In mice, these effects included increased mortality (at high doses), decreased body weight gain, increased serum glucose, increased kidney weights, cystic thyroid follicles, and increased hepatocellular adenomas and possibly hepatocellular carcinomas in males (but not in females).

In an acute oral neurotoxicity study in rats, the acute general systemic toxicity LOAEL was 1000 mg/kg based on soft stools and decreased motor activity on the day of dosing. These effects were considered to be a manifestation of acute general systemic toxicity and not a direct neurotoxic response to the administration of the test material. The acute general systemic toxicity NOAEL was 50 mg/kg. The LOAEL for neurotoxic effects was not identified (>2000 mg/kg). The NOAEL for neurotoxic effects was 2000 mg/kg.

Of particular concern was a neurotoxic lesion described as vacuolation of the white matter of the central nervous system (CNS) which was observed initially in long-term (1-2 year) chronic studies on mice and dogs and later, upon careful re-examination of the CNS, also in shorter-term (4-week to 90-day) subchronic studies on mice and dogs. This lesion was observed during the (light) microscopic examination of several tissues of the CNS and occurred most frequently in brain (sections of cerebrum and/or sections of cerebellum, pons, medulla, midbrain) and less frequently in cervical spinal cord. Although this lesion was also observed in control animals, the increased

incidence and/or severity of the lesion in test animals was clearly treatment-related and dose-related. Further investigation of this lesion in a series of special studies demonstrated the same lesion could also be induced in rats. In the special studies, the following was also determined.

- (1) Fluazinam, per se, was not responsible for the induction of this lesion. An analysis of the effects of impurities present in technical grade fluazinam revealed that one single impurity, Impurity-5, was solely responsible for the appearance of white matter vacuolation.
- (2) No significant differences in susceptibility or in incidence or severity of vacuolation of the white matter of the CNS were observed between species (mice, dogs, or rats). Similarly, no significant differences were attributed to sex.
- (3) White matter vacuolation in the CNS was reversible. Electron microscopy of the white matter (cerebellum) of mice treated with technical grade fluazinam indicated that treatment-related effects were confined to the myelin sheaths. Large vacuoles were observed in the intramyelin sheaths due to the accumulation of fluid between the sheaths. The nucleus and mitochondria in oligodendroglia were observed to remain intact, suggesting no damage to these cells. The myelin sheaths appeared to recover completely during a recovery period of up to 56 days.
- (4) There appears to be a non-linear dose-response with a clear threshold below which no effect occurs. It was concluded that a LOAEL of 0.1 mg/kg/day and a NOAEL of 0.02 mg/kg/day for CNS effects could be established for Impurity-5.

Based on a consideration of all the available data and information relating to this treatment-related neurotoxic lesion, it was concluded that the chronic RfD of 0.011 mg/kg/day for the general population (including infants and children) is protective of the CNS effects caused by Impurity-5 present in technical grade fluazinam.

Combined results from two carcinogenicity studies in mice indicated that treatment of mice for up to 104 weeks resulted in statistically significant increases in the incidence of hepatocellular adenoma in male mice at 1000 and 3000 ppm. These increased incidences slightly exceeded the upper range of the historic control incidences. The hepatocellular carcinoma incidence was also increased in male mice at these same dose levels, but was not statistically significant and was within the range of historic control incidences. The incidences of combined hepatocellular adenoma and/or carcinoma were also increased in male mice at 1000 ppm, 3000 ppm ($p < 0.01$) and 7000 ppm ($p < 0.05$). No significant increases in liver tumors were seen in treated female mice compared to controls. In a combined chronic toxicity/carcinogenicity study in rats, a slightly increased incidence of thyroid gland follicular cell adenomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male rats at 100 ppm and 1000 ppm. Also, a slightly increased incidence of thyroid gland follicular cell adenocarcinomas, which exceeded the upper range of historical control data for the same tumor type, was observed in male

rats at 1000 ppm. In a battery of mutagenicity studies on technical grade fluazinam, results in all studies were negative for mutagenic potential.

In a developmental toxicity study in rats, the maternal toxicity LOAEL was 250 mg/kg/day based on decreased body weight gain, decreased food consumption, increased water consumption, and increased urogenital staining during treatment. The maternal toxicity NOAEL was 50 mg/kg/day. The developmental toxicity LOAEL was 250 mg/kg/day based on decreased fetal body weights; decreased placental weights; increased fetal incidences of facial/palate clefts, diaphragmatic hernia and delayed ossification in several bone types; greenish amniotic fluid and possible increased late resorptions and postimplantation loss. The developmental toxicity NOAEL was 50 mg/kg/day. Although quantitative evidence of increased susceptibility of fetuses to fluazinam was not observed, the increased fetal incidences of facial/palate clefts and other rare deformities observed in this study were considered to be qualitative evidence of increased susceptibility of fetuses to fluazinam.

In a developmental toxicity study in rabbits, The maternal toxicity LOAEL was 7 mg/kg/day based on decreased food consumption and increased liver histopathology. The maternal toxicity NOAEL was 4 mg/kg/day. The developmental toxicity LOAEL was 12 mg/kg/day based on an increased incidence of total litter resorptions and a possibly increased incidence of fetal skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternbrae, and abnormalities of head bones. The developmental toxicity NOAEL was 7 mg/kg/day. It was determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to *in utero* exposure to fluazinam was observed in this study.

In a 2-generation reproduction study in rats, The NOAEL for parental toxicity was 20 ppm (1.9 mg/kg/day) and the LOAEL was 100 ppm (9.7 mg/kg/day), based on liver pathology (increased incidences of periacinar hepatocytic fatty changes) in F₁ males. The NOAEL for reproductive toxicity was 100 ppm (10.6 mg/kg/day) and the LOAEL was 500 ppm (53.6 mg/kg/day), based on a decreased number of implantation sites and decreased litter sizes to day 4 post partum for F₁ females (F₂ litters). The NOAEL for developmental toxicity was 100 ppm (8.4 mg/kg/day) and the LOAEL was 500 ppm (42.1 mg/kg/day), based on decreased body weight gain during lactation for both F₁ and F₂ pups. It was determined that neither quantitative nor qualitative evidence of increased susceptibility of fetuses to fluazinam was observed in this study.

In a metabolism study in rats, only 33-40% of the administered dose of radiolabeled fluazinam was absorbed. Most of the administered dose was recovered in the feces (>89%). Identified fecal metabolites represented only 11-68% of the administered dose. Unabsorbed parent compound represented most of the identified radioactivity in the feces. Most of the remaining fecal metabolites appeared in unextractable components in the post-extraction solid. Excretion via the urine was minor (<4%). Total biliary radioactivity, however, represented 25-34% of the administered dose, indicating considerable enterohepatic circulation. Analysis of chromatograms indicated that numerous metabolites were present in the bile. Metabolite profiles from

administration of different label positions (pyridyl and phenyl) indicated that there was no metabolic cleavage of the ring structures in parent fluazinam.

7 **DATA GAPS**

The scientific quality and completeness of the available toxicology data base are considered adequate according to the Subdivision F Guidelines and Part 158 data requirements to support the registration of this food-use chemical (except as described below). At this time, there is high confidence in the hazard endpoints and dose-response assessments conducted for this chemical.

Mutagenicity Study: Mouse Lymphoma Assay § 870.5300

The applicant has informed the Agency that it intends to perform and submit a new mouse lymphoma assay by November, 2000.

28-Day Inhalation Study in Rats See § 870.3465

Developmental neurotoxicity study, rats § 870.6300

Test Material: to be technical grade fluazinam containing maximum level of Impurity-5 permitted in specification for technical grade fluazinam (currently 0.3%, but may be reduced to 0.1%. See letter from Gary L. Eilrich, Vice President, Regulatory Affairs, ISK Biosciences Corporation to Mr. Jim Jones, Director, RD, OPP, dated August 3, 2000).

Proposed Protocol: to include full neurohistopathological examination of dams. Protocol to be submitted to EPA for comment prior to commencement of study.

Based on a consideration of the results in the developmental neurotoxicity study in rats required above, HIARC will subsequently determine whether a repeat of the subchronic neurotoxicity study in rats (§ 870.6200) will also be required to support the registration of fluazinam.

Subchronic neurotoxicity study, rats § 870.6200

Requirement: reserved; to be determined at a later time (see above).

8 Acute Toxicity of Technical Grade Fluazinam

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral Rats lot # 109 (95.3%)	42248603	M: LD ₅₀ = 4500 mg/kg F: LD ₅₀ = 4100 mg/kg	III
81-1	Acute Oral Rats lot #8412-20 (95.3%)	42248602	M: LD ₅₀ > 5000 mg/kg F: LD ₅₀ > 5000 mg/kg	IV
81-1	Acute Oral Rats lot #1/87 (97.9%)	42248604	M: LD ₅₀ > 5000 mg/kg F: LD ₅₀ > 5000 mg/kg	IV
81-2	Acute Dermal Rats lot # 8303-2 (98.5%)	42248605	M: LD ₅₀ >2000 mg/kg F: LD ₅₀ >2000 mg/kg	III
81-3	Acute Inhalation Rats lot # 109 (95.3%)	42270601	M: LC ₅₀ = 0.463 mg/L F: LC ₅₀ = 0.476 mg/L	II
81-4	Primary Eye Irritation Rabbits lot # SNPE B-1216, No. 1006 (97.9%)	42248606	Extremely irritating. Corneal opacity did <u>not</u> reverse in 21 days	I
81-5	Primary Skin Irritation Rabbits lot # SNPE B-1216, No. 1006 (97.9%)	42248607	Slight dermal irritant	IV
81-6	Dermal Sensitization Guinea Pigs lot # 1030/91 (96.7%)	42274401	<u>POSITIVE</u> for dermal sensitization	N/A
81-6	Dermal Sensitization Guinea Pigs Ultra-purified fluazinam (100%)lot #Y910401	42248608	<u>NEGATIVE</u> for dermal sensitization	N/A

Acute Toxicity of Formulated Product (Omega 500F)

All studies conducted on Omega 500F (EPA file symbol 71512-R), 40% fluazinam, yellow liquid.

GDLN	Study Type	MRID	Results	Tox Category
81-1	Acute Oral Rats	42974907	M: LD ₅₀ > 5000 mg/kg F: LD ₅₀ > 5000 mg/kg	IV
81-2	Acute Dermal Rabbits	42974908	M: LD ₅₀ > 2000 mg/kg F: LD ₅₀ > 2000 mg/kg	III
81-3	Acute Inhalation Rats	42311001	M: LC ₅₀ = 3.0 mg/L F: LC ₅₀ = 3.4 mg/L	IV *
81-4	Primary Eye Irritation Rabbits	42974910	Slightly irritating	III
81-5	Primary Skin Irritation Rabbits	42974911	Moderately irritating	II
81-6	Dermal Sensitization Guinea Pigs	42974912	<u>POSITIVE</u> for dermal sensitization	N/A

- Based on results of acute inhalation toxicity study on rats on Fluazinam 50% WP (51.3% fluazinam) (MRID 42311001) in which LC₅₀ = 3.0 mg/L for males and LC₅₀ = 3.4 mg/L for females, Toxicity Category IV. See memorandum by Byron T. Backus, Technical Review Branch, RD, dated April 3, 2000, for more detailed information and rationale.

9 SUMMARY OF TOXICOLOGY ENDPOINT SELECTION

The doses and toxicological endpoints selected for various exposure scenarios are summarized below.

EXPOSURE SCENARIO	DOSE (mg/kg/day)	ENDPOINT	STUDY
Acute Dietary (Females 13-50 yrs)	Developmental NOAEL = 7 UF = 100	Increased incidence of total litter resorptions and possibly increased incidence of fetal skeletal abnormalities	Developmental toxicity-rabbits
	Acute RfD = 0.07 mg/kg/day		
Acute Dietary (General Population including Infants & Children)	Systemic Toxicity NOAEL = 50 UF = 100	Decreased motor activity and soft stools on day of dosing	Acute neurotoxicity-rats
	Acute RfD = 0.50 mg/kg/day		
Chronic Dietary (General Population including Infants & Children)	NOAEL = 1.1 UF = 100	Liver histopathology and increased liver weight	Carcinogenicity-mice
	Chronic RfD = 0.011 mg/kg/day		
Oral, Incidental Short-Term Intermediate-Term	Maternal NOAEL = 4 MOE = 100	Liver histopathology and decreased food consumption	Developmental toxicity-rabbits
Dermal Short-Term Intermediate-Term	Systemic Toxicity NOAEL = 10 MOE = 100	Increased cholesterol, increased aspartate aminotransferase (target organ: liver)	21-Day dermal-rats
Dermal Long-Term ⁽¹⁾	NOAEL = 1.1 MOE = 100	Liver histopathology and increased liver weight	Carcinogenicity-mice
Inhalation Short-Term	NOAEL = 1.38 (NOAEL adjusted for 51.9% a.i.) MOE = 300	Increased liver weights, increased testes weights	7-Day inhalation-rats (Test material: Frowncide WP containing 51.9% a.i.)
Inhalation Intermediate-Term Long-Term	NOAEL = 1.38 (NOAEL adjusted for 51.9% a.i.) MOE = 1000	Increased liver weights, increased testes weights	7-Day inhalation-rats (Test material: Frowncide WP containing 51.9% a.i.)
Carcinogenicity Chronic Exposure	Referred to Carcinogenicity Assessment Review Committee (10/24/00)	Increased incidence of hepatocellular adenomas and combined hepatocellular adenomas/carcinomas in male mice. Possibly increased incidence of thyroid gland follicular cell adenomas and adenocarcinomas in male rats	Carcinogenicity, mice and Chronic toxicity/carcinogenicity, rats

⁽¹⁾ To evaluate the long-term dermal risk, a dermal absorption factor of 25% should be applied to the NOAEL of 1.1 mg/kg/day.