UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

August 22, 2007

MEMORANDUM

SUBJECT: Fluazinam: Human Health Risk Assessment for Proposed Use on Edible-Podded Beans, Shelled Succulent and Dried Beans, *Brassica* Leafy Vegetables, Bushberries, and Ginseng. PC Code: 129098, Petition No: 6E7139, DP Barcode: D334949

Regulatory Action: Section 3 Registration Action
Risk Assessment Type: Single Chemical Aggregate

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1.0 Executive Summary

The active ingredient (ai), fluazinam, is a preventive contact fungicide with a multi-site mode of action. It disrupts the production of energy at several metabolic sites within the fungal cell. Interregional Research Project #4 (IR-4) has submitted petitions (PPs#6E7137, 6E7139) proposing the use of a formulation on ginseng, dry beans and succulent bean crops, edible-podded legume vegetables, bushberry and *Brassica* leafy vegetables. ISK Biosciences Corporation is the data submitter and registrant for the active ingredient (ai), fluazinam, in the US. The following products have been assessed for occupational exposure: OMEGA® 500F and Allegro® 500F. The products are formulated as a flowable suspension/liquid containing 40.0% fluazinam and may be applied by airblast or groundboom (aerial application of this EP is prohibited). Based on the anticipated application practices for the OMEGA® 500F and Allegro® 500F Fungicide, product labels and information provided by the registrant, handler exposures are expected to be short- and intermediate-term in duration.

HUMAN HEALTH RISK ASSESSMENT:

Toxicology/Hazard

In subchronic and chronic oral and dermal studies in rats, dogs and mice, the liver appeared to be the primary target organ. Signs of liver toxicity included: changes in clinical chemistry (e.g. increased serum alkaline phosphatase, increased aspartate aminotransferase), increased absolute and/or relative liver weights, increased incidences of gross lesions (e.g. pale, enlarged, pitted, mottled, accentuated markings) and increased incidences of a variety of histopathological lesions.

Treatment-related effects were also observed in other organs in subchronic and chronic oral, dermal and inhalation studies in rats, dogs and mice, but these effects were not regularly noted in all three species or in all studies in a given species. In rats, effects observed were decreased body weight gain, decreased food consumption, mild anemia, increased serum cholesterol, increased serum phospholipid, increased serum aspartate aminotransferase, testicular atrophy, increased testes weights (inhalation study), pancreatic exocrine atrophy, increased lung weights, increased alveolar adenomatosis, epithelialization and macrophages, thyroid gland follicular cell hyperplasia, and an increased incidence of thyroid gland follicular cell tumors in male rats, but not in female rats. 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 follicules, and an increased incidence of both benign and malignant hepatocellular liver tumors in male mice.

In a developmental toxicity study in rats there was evidence of increased qualitative susceptibility (skeletal abnormalities/facial/palate clefts in fetuses vs. decreases in bodyweight gain/food consumption in maternal animals) of fetuses to fluazinam; there was no evidence of increased quantitative susceptibility. There was no evidence of increased quantitative or qualitative susceptibility in a developmental toxicity study in rabbits or a 2-generation reproduction study in rats. Effects included: increased incidences of total litter resorptions and

fetal skeletal abnormalities (eg. kinked tail tip, fused or incompletely ossified sternebrae, and abnormalities of head bones) in rabbits and decreased body weight gain in rats. Reproductive effects were a decreased number of implantation sites and decreased litter size.

In an acute neurotoxicity study in rats, there were decreases in motor activity and soft stools at high doses (1000 mg/kg/day). In two subchronic neurotoxicity studies (evaluated together) there were no signs of neurotoxicity observed up to 280 mg/kg/day. A neurotoxic lesion described as vacuolation of the white matter of the central nervous system was observed in studies in mice and dogs; this lesion was found to be reversible and is attributed to an impurity (impurity-5).

A developmental neurotoxicity study in rats and a series of special studies were submitted to address the issues of increased susceptibility and the presence of neurotoxic lesions observed in the toxicological database. As a result, there are no residual uncertainties with regard to pre- and/or postnatal toxicity and no additional factors are needed. Additionally, HED recommends the FQPA SF be reduced to 1X because the toxicology database is complete in regard to pre-and postnatal toxicity and neurotoxicity; the dietary food exposure assessment is based on HED-recommended tolerance-level residues and assumes 100% crop treated for all commodities, resulting in upper bound estimates of dietary exposure; the drinking water assessment is based on values generated by model and associated modeling parameters which are designed to provide conservative, health protective upper bound estimates of water concentrations; and there are no registered or proposed residential uses.

The Cancer Assessment Review Committee (CARC) classified fluazinam as "Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential," and determined that quantification of human cancer risk is not required.

For acute dietary exposure (females 13-49), the developmental toxicity study in rabbits was used to calculate the acute reference dose (aRfD) of 0.07 mg/kg/day. The developmental NOAEL of 7 mg/kg/day and the LOAEL of 12 mg/kg/day were based on increased incidences of total litter resorptions and slight increased incidences of fetal skeletal abnormalities. The aRfD of 0.5 mg/kg/day calculated for general population acute dietary exposure was based on a LOAEL of 1000 mg/kg/day (NOAEL=50 mg/kg/day) from an acute neurotoxicity study in rats; adverse effects seen were decreases in motor activity and soft stools on the day of dosing. For chronic dietary exposure (all populations), the carcinogenicity study in mice was used to calculate the chronic reference dose (cRfD) of 0.011 mg/kg/day. The NOAEL of 1.1 mg/kg/day and the LOAEL of 10.7 were based on adverse liver alterations (increased liver weights and histopathology). A 21-day dermal toxicity study in rats was used to select the dose and endpoint for occupational short- and intermediate-term dermal exposure. The NOAEL of 10 mg/kg/day and the LOAEL of 100 mg/kg/day were based on increased cholesterol and aspartate aminotransferase. For occupational short- and intermediate-term inhalation exposure, a 7-day inhalation study in rats was used. The NOAEL of 1.38 and the LOAEL of 3.87 mg/kg/day were based on increased liver weights and testes weights in males. In the inhalation study, a histopathological examination was not performed; thus an additional factor of 10x was applied to the conventional uncertainty factor of 100x. This factor also addresses the use of a short-term (7 days) study to evaluate intermediate-term inhalation exposure. There are no residential uses proposed for fluazinam; therefore, incidental oral and residential dermal and inhalation risk assessments were not conducted.

Dietary Exposure (Food/Water)

HED performed both the acute and chronic analyses that are based on tolerance-level residues, assume 100% crop treated, and incorporate modeled estimated drinking water concentrations (EDWCs). Therefore, the resulting exposure and risk estimates should be considered high-end and very conservative.

The acute risk estimates are below HED's level of concern for all population subgroups, including those of infants and children. Generally, HED is concerned when risk estimates exceed 100% of the population-adjusted dose (PAD). The acute risk estimate for the U.S. population, as a whole, is 1% of the acute PAD (aPAD). For females 13-49 years of age, the risk estimate is 8% of their aPAD. Risk estimates for all other population subgroups are less than 8% aPAD. Likewise, chronic risk estimates are below HED's level of concern for all population subgroups. The risk estimate for the U.S. population is 9% of the chronic PAD (cPAD). The highest risk estimate is for All Infants (< 1 year) population subgroup at 16% cPAD.

The nature of the residue in plants has been adequately delineated, based on acceptable potato, peanut, and grape metabolism studies reviewed previously (D257115; William Cutchin; 5/21/2001), along with an acceptable apple metabolism study submitted recently (MRID# 46991301). At a meeting held on 11/28/2000, HED concluded that the residue of concern (ROC) in potatoes and peanuts (for both tolerance expression and dietary risk assessment purposes) was the parent compound only (D272624; William Cutchin; 4/23/2001). In wine grapes, both parent and AMGT were included in the ROC for tolerance expression and risk assessment. Additionally, HED determined that data generated for potential new uses on other crops (with the exception of root and tuber, and bulb vegetables) should include analyses for both parent and AMGT; thus, the ROC for the proposed primary crops are parent and AMGT.

The nature of the residue in livestock is also understood, based on adequate goat and hen metabolism studies (D257115; William Cutchin; 5/21/2001). The fluazinam residues of regulatory interest in animals were determined by HED to be parent plus the metabolites AMPA and DAPA, and their sulfamate conjugates.

The submitted gas chromatography with electron-capture detection (GC/ECD) methods (modifications of the tolerance-enforcement method) are adequate for collecting data and enforcing tolerances for residues of fluazinam *per se* in the various crop commodities associated with these petitions. The tolerance-enforcement method, *Fluazinam: Method for the Analysis in Peanut Nut Meat* (MRID #43521016), was adequately radiovalidated, and underwent a successful independent laboratory validation (ILV) trial. The method was forwarded to BEAD's Analytical Chemistry Branch (ACB) for a petition method validation (PMV) trial, and was subsequently determined to be suitable as a tolerance-enforcement method (D266802; Paul Golden; 6/22/2001).

The submitted high performance liquid chromatography with ultraviolet detection (HPLC/UV) method (a working method based on *Method Evaluation for the Analysis of AMGT in Grapes*, MRID #45593101) is adequate for collecting data on AMGT residues in blueberries. The LLMV, limit of detection (LOD), and LOQ were 0.020, 0.013, and 0.038 ppm, respectively, for residues of AMGT in blueberries. HED has previously determined that residues of AMGT are to

be regulated in wine grapes (D272624; William Cutchin; 4/23/2001). The Agency therefore requested that this method undergo an ILV trial, and, potentially, a PMV trial by the ACB. An ILV study has not yet been submitted.

The multiresidue method (MRM) testing data indicate that fluazinam is partially recovered through Sections 302, 303, and 304 of PAM Volume I, with its recovery being dependent on which Florisil elution system is used. The MRMs can serve as a confirmatory procedure for residues of fluazinam. Data should also be provided for the metabolite AMGT, since it is included in the tolerance expression for grapes.

Adequate storage stability data were collected indicating that fluazinam residues were stable under frozen storage in blueberries, snap beans, and broccoli for the storage durations and conditions of the samples from the respective crop field trials. In blueberries, AMGT residues were stable under frozen storage for the storage durations and conditions of the samples from the blueberry field trials. However, storage stability studies indicated that there was significant dissipation of fluazinam residues under frozen storage in ginseng, lima beans, dried beans, cabbage, and mustard greens. Correction factors were therefore incorporated into the recommended tolerances for fluazinam in ginseng, shelled succulent beans, and shelled dried beans to account for dissipation during storage. A correction factor was not utilized when setting the recommended tolerance in *Brassica* leafy vegetables, because fluazinam applications made to cabbage and mustard greens were essentially identical to the treatment of broccoli (which had acceptable storage stability), and all residues in treated samples from the *Brassica* field trials were ≤ LOQ (≤ 0.010 ppm).

The available crop field trial data are adequate, and support the proposed uses. However, it was noted that residue data for AMGT were provided only for blueberries; AMGT data should also have been included with the field trial studies for edible-podded beans, shelled succulent and dried beans, and *Brassica* vegetables.

There are no processed commodities for which residue data are required associated with the proposed uses on the crops requested in the subject petitions under review.

There are no significant livestock feed items associated with the proposed uses on the crops requested in the subject petitions under review.

Regulatory requirements pertaining to fluazinam residues in rotational crops have been fulfilled, and the rotational crop restrictions on the proposed label are adequate.

There are no established or proposed Canadian or Codex Maximum Residue Limits (MRLs) for residues of fluazinam in plant or animal commodities. There are Mexican MRLs established for residues of fluazinam in potato at 0.05 ppm, and in beans at 0.1 ppm.

Occupational Exposure/Risk

Handlers

No chemical-specific data for assessing handler exposures were submitted to the Agency in support of the proposed uses. As a result, HED used surrogate data from the Pesticide Handlers Exposure Data Base (PHED) Version 1.1, and standard values established by the Health Effects Division (HED) Science Advisory Council for Exposure, for acres treated per day, body weight, and the level of personal protective equipment (PPE) worn by handlers. HED's level of concern (LOC) for occupational dermal exposures is 100 (i.e., MOE less than 100 is of concern). The level of concern for inhalation exposures is 1000 (i.e., MOE less than 1000 is of concern).

All dermal risk estimates for short- and intermediate-term handler exposure resulted in MOEs greater than 100 with the use of gloves. All inhalation risk estimates for short- and intermediate-term handler exposure resulted in MOEs greater than 1000 with the use of a dust mist respirator. Summaries of the dermal and inhalation (MOEs) short- and intermediate-term risks for handlers are provided in Table 9.1.2.

Postapplication

Chemical-specific postapplication data were submitted in support of this registration action. For purposes of comparison, a Tier 1 (HED standard assumptions and defaults of 20% DFR) and Tier 2 (chemical-specific foliar residue data) analysis were performed to ensure that potential postapplication exposures are not of concern. A comparison of Tier 1 and Tier 2 analyses resulted in similar postapplication exposure risks of concern (MOE < 100).

Since the Tier 1 and Tier 2 analyses resulted in similar exposures and risks , HED based its postapplication assessment on the Tier 2 analysis. The only crop scenarios which resulted in MOEs greater than 100 on day 0 (immediately after application) were for low exposure activities (i.e., scouting, hand weeding, thinning and irrigation) for beans and ginseng. All other crops (i.e. bushberries, *Brassica* and leafy vegetables) did not reach a MOE greater than or equal to 100 for low exposure activities until 3 to 13 days later. All medium (i.e., scouting, hand weeding, and irrigation) and high (i.e., hand harvesting/ pruning/pinching/training) postapplication exposure activities for all crops resulted in MOEs below 100 on day of application. Crops did not reach MOEs greater than or equal to 100 until 4 to 20 days later depending on the specific crop.

Since postapplication exposure resulting in MOEs below 100 may be an indication of possible risk for re-entry of workers, HED provided a comparison of the estimated number of days required before a MOE of 100 is reached (i.e. restricted entry interval – REI) based on Tier 2 analysis to establish pre-harvest interval (PHI). **HED recommends that the Registration Division ensure that the PHIs do not go below the calculated REIs for harvesting**.

The Tier 2 postapplication estimates of exposure may be overestimating residues on the proposed crops based on the methodology used to determine dislodgeable residues. However, HED cannot refine these estimates without chemical specific data collected in accordance with Agency guideline methods. A possible option for the registrant would be to repeat the DFR studies using guideline methods (i.e., leaf punch and dislodgeable residues with surfactant as opposed to whole leaf extraction).

Restricted Entry Interval

The technical material has an Acute Eye Irritation Toxicity Category I. Per the Worker Protection Standard (WPS), a 48-hr restricted entry interval (REI) is required for chemicals classified under Toxicity Category I. The 48 hour REI appearing on the label is only appropriate for postapplication activities for which the MOE reaches 100 on day 0. However, note that an interval of 3 to 20 days is necessary to reach a MOE of 100 for medium and high postapplication exposure activities (i.e., hand weeding/harvesting/pruning/pinching/training, and irrigation). **HED recommends that the proposed label be revised to ensure that the appropriate REI restrictions are clearly stated for all crops and do not exceed pre-harvest intervals.**

ENVIRONMENTAL JUSTICE CONSIDERATIONS

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations" http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf).

As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intakes by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas postapplication are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

Review of Human Research

This risk assessment relies in part on data from studies in which adult human subjects were intentionally exposed to a pesticide or other chemical. These studies, which comprise the Pesticide Handlers Exposure Database (PHED), have been determined to require a review of their ethical conduct, and have received that review. The studies in PHED were considered appropriate (ethically conducted) for use in risk assessments.

ADDITIONAL DATA NEEDS/RECOMMENDATIONS

Toxicology

A 28-day subchronic inhalation study is recommended to support the registration of fluazinam. If an acceptable subchronic inhalation study is submitted and determined to be more appropriate for endpoint selection, the additional 10x safety factor can be reduced.

Residue Chemistry

No major deficiencies were noted in the subject petition that would preclude the establishment of permanent tolerances for fluazinam residues in the requested crops. Revised Sections F should be submitted, so that the proposed tolerances reflect the recommended tolerance levels, and correct commodity definitions, as specified in Appendix C. Issues pertaining to residue chemistry deficiencies should be resolved (see below).

Regulatory Recommendations

As a condition of registration, results of an ILV trial for the AMGT analytical method (with wine grapes) should be submitted by the registrant. If the registrant agrees with the modifications made by Ricerca to the original method (in MRID #45593101), these modifications should be incorporated into a revised method for the ILV. Sample sets should include, at the minimum, 2 control (untreated) samples of wine grapes, 2 samples fortified at the tolerance level (3.0 ppm), and 2 samples fortified at the LOQ (0.010 ppm).

As a condition of registration, MRM recovery data should be provided for the metabolite AMGT, since it is included in the tolerance expression for wine grapes.

The product label for Omega 500F should be amended to include a restriction, stating that turnip roots from turnip plants treated with this EP must not be used for human nor livestock consumption.

The Agency has previously determined, and the registrant is hereby advised again, that residue data for AMGT should be provided in the crop field trial studies for all future requested plant commodities, except root and tuber, and bulb vegetables.

HED recommends in favor of establishing permanent tolerances for fluazinam in the requested crops, at the levels specified in Appendix C (Table C.1. Tolerance Summary of Fluazinam).

Occupational and Residential Exposure

HED recommends that the Registration Division ensure that the PHIs do not go below calculated REIs for harvesting. Additionally, HED recommends that the proposed label be revised to ensure that the appropriate REI restrictions are clearly stated for all crops and correspond to the postapplication activities and reentry intervals.

2.0 Ingredient Profile

Fluazinam (Omega 500F Agricultural Fungicide) is a non-systemic, preventive, contact fungicide of the phenyl-pyridinamine class, with a multi-site mode of action. It disrupts the production of energy at several metabolic sites within the fungal cell. Fluazinam is a protectant fungicide; when applied to plants, it remains primarily on the plant surface, is not taken up to any extent by the plant, and is not translocated within the plant.

2.1 Summary of Registered/Proposed Uses

Fluazinam is currently registered for use on peanuts and potatoes. There is also a tolerance established for fluazinam in imported wine grapes (without US registration). Permanent tolerances are established for residues of fluazinam in peanuts and potatoes at 0.02 ppm (40CFR §180.574[a][1]), and in imported wine grapes at 3.0 ppm (40CFR §180.574[a][2]). Interregional Research Project #4 (IR-4) has submitted petitions (PPs#6E7137, 6E7139) proposing the use of a formulation containing 4.17 pounds per gallon (lb/gal) of fluazinam (Omega 500F Agricultural Fungicide; EPA Registration #71512-1) on various crops. This end-use product (EP) is formulated as a flowable-suspension (F) concentrate. ISK Biosciences Corporation is the data submitter and registrant for the active ingredient (a.i), fluazinam, in the US. Copies of the proposed labels were provided, and the proposed uses on the requested crops are summarized in Table 2.1 (below). Applications of Omega 500F are to be made using ground equipment or chemigation (application via irrigation equipment) only; aerial application of this EP is prohibited.

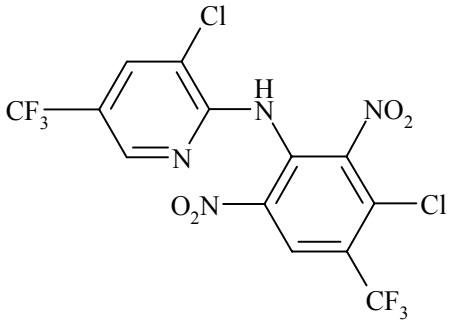
TABLE 2.1 Summary of Directions for Use of Fluazinam.

Application Timing; Type; and Equipment [1]	Form-ulation [2]	Use Rate (lb ai/A)	Maximum # of Uses per Season	Maximum Seasonal Use Rate (lb ai/A)	PHI (Days)	Use Directions and Limitations
Shelled Succulent and Dried Beans						
At 10-30% bloom; foliar; spray.	Omega 500F	0.26-0.45	2	0.90	30	RTI = 7-10 days. Volume adequate to cover foliage and flowers.
Ginseng						
At transplant (for root rot); broadcast; spray.	Omega 500F	0.52	6	3.1	30	RTI = 14 days. Spray volume ≥ 100 gal/A.
At disease appearance or favorable conditions (for blight/white mold); broadcast; spray.		0.52-0.78	4-6			RTI = 7-14 days. Spray volume ≥ 100 gal/A.
Edible-Podded Beans						
At 10-30% bloom; foliar; spray.	Omega 500F	0.26-0.45	2	0.90	14	RTI = 7-10 days. Volume adequate to cover foliage and flowers.
***Brassica* (Cole) Vegetables**						
At transplant; soil drench; spray.	Omega 500F	0.055 lb ai/1000 plants	1	2.0	20/50 [3]	6.45 oz EP/100 gal water, 3.4 oz (100 mL)/plant
Prior to transplant; soil incorporation; precision incorporator.		1.36				Band width ≥9", soil depth 6-8". Spray volume ≥ 50 gal/A.
Prior to forming bed; broadcast; spray.		2.0				Spray volume ≥ 50 gal/A.
Bushberries						

TABLE 2.1 Summary of Directions for Use of Fluazinam.						
Application Timing; Type; and Equipment [1]	**Form-ulation [2]**	**Use Rate (lb ai/A)**	**Maximum # of Uses per Season**	**Maximum Seasonal Use Rate (lb ai/A)**	**PHI (Days)**	**Use Directions and Limitations**
1Green tip, **2**pink tip, **3**early bloom, **4**full bloom, **5**blossom drop, **6**small green fruit/some blue fruit; foliar; spray.	Omega 500F	0.65	6	3.9	30	RTI = 7-10 days. Volume adequate to cover foliage, flowers, and fruit.

1. Applications of Omega 500F are to be made using ground equipment or chemigation (application via irrigation equipment) only. Aerial application of this EP is prohibited.
2. Omega 500F is a flowable suspension concentrate containing 4.17 lb/gal of fluazinam.
3. PHI = 20 days for *Brassica* leafy greens, and 50 days for *Brassica* heading vegetables.

2.2 Structure and Nomenclature

TABLE 2.2 Test Compound Nomenclature.	
Compound	Chemical Structure 
Empirical Formula	$C_{13}H_4Cl_2F_6N_4O_4$
Molecular Weight	465.1
Common Name	Fluazinam
Company Experimental Names	Fluazinam, IKF-1216
IUPAC Name	3-chloro-*N*-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine
CAS Name	3-chloro-*N*-[3-chloro-2,6-dinitro-4-(trifluoromethyl)phenyl]-5-(trifluoromethyl)-2-pyridinamine
CAS Number	79622-59-6
End-use Product/(EP)	Omega® 500F (USA); Allegro® 500F (Canada)

2.3 Physical and Chemical Properties

TABLE 2.3 Physicochemical Properties of Fluazinam		
Parameter	**Value**	**Reference**
Melting Point/Range	115-117°C	The e-Pesticide Manual (13th Edition) Version 3.1
pH	5.85	MRID #43521001
Density (25°C)	1.02 g/cm^3*	LSS 2000_1973_2LS_rev

TABLE 2.3 Physicochemical Properties of Fluazinam			
Parameter	**Value**		**Reference**
Water Solubility (25°C)	(pH buffered to 5) 0.131 mg/L (pH buffered to 7) 0.157 mg/L (pH buffered to 9) 3.384 mg/L		LSS 2000_1973_2LS_rev
Solvent Solubility (25°C)	Solvent	Solubility (mg/mL)	LSS 2000_1973_2LS_rev
	acetone dichloromethane ethyl acetate ethyl ether hexane methanol octanol toluene	853 675 722 231 8 192 41 451	
Vapor Pressure	Temp (°C)	Vap. Press. (Pa)	LSS 2000_1973_2LS_rev
	25 35 45	2.3×10^{-5} 1.3×10^{-4} 6.7×10^{-5}	
Dissociation Constant (pK_a)	Average pKa = 7.22 in 50% ethanol/water (v/v)		LSS 2000_1973_2LS_rev
Octanol/Water Partition Coefficient (Log [K_{OW}])	1.08×10^4 (Log K_{ow} = 4.03)		LSS 2000_1973_2LS_rev
UV/Visible Absorption Spectrum	pH	λ_{max} (nm)	Regulatory Note REG2003-12
	5 7 >10	238 239, 342 260, 343, 482	

*REG2003-12 states the relative density as 1.76 g/cm^3, temperature not stated.

3.0 Hazard Characterization/Assessment

3.1 Hazard and Dose-Response Characterization

3.1.1 Database Summary

3.1.1.1 Sufficiency of studies/data

Based on the proposed use pattern, the toxicology database for fluazinam is complete and adequate for risk assessment. There are acceptable studies available for endpoint selection that include: 1) subchronic oral toxicity studies in rats, mice, and dogs; 2) a chronic oral toxicity study in dogs and carcinogenicity studies in rats and mice; 3) developmental and reproduction studies in rats and a developmental study in rabbits; and 4) a subchronic dermal toxicity study in rats. There is also a complete mutagenicity battery, acute LD50 and neurotoxicity studies (acute, subchronic, and developmental), as well as a metabolism study in the rat.

3.1.1.2 Mode of action

Fluazinam is a preventive contact fungicide with a multi-site mode of action. It disrupts the production of energy at several metabolic sites within the fungal cell. Fluazinam is a protectant

fungicide; when applied to plants, it remains primarily on the plant surface. It is not taken up to any extent by the plant, and is not translocated within the plant like systemic fungicides.

3.1.2 Toxicological Effects

In subchronic and chronic oral and dermal studies in rats, dogs and mice, the liver appeared to be a primary target organ. Signs of liver toxicity included: changes in clinical chemistry (e.g. increased serum alkaline phosphatase, increased aspartate aminotransferase), increased absolute and/or relative liver weights, increased incidences of gross lesions (e.g. pale, enlarged, pitted, mottled, accentuated markings), and a variety of histopathological 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 mice, but these effects were not regularly noted in all three species or in all studies in a given species. In rats, effects observed were decreased body weight gain, decreased food consumption, mild anemia, increased serum cholesterol, increased serum phospholipid, increased serum aspartate aminotransferase, testicular atrophy, increased testes weights (inhalation study), pancreatic exocrine atrophy, increased lung weights, increased alveolar adenomatosis, epithelialization and macrophages, thyroid gland follicular cell hyperplasia, and an increased incidence of thyroid gland follicular cell tumors in male rats, but not in female rats. In dogs, effects included increased salivation, increased nasal dryness, grey mottling of the retina, mild anemia, increased serum alkaline phosphatase and gastric lymphoid hyperplasia. In mice, increased mortality (at high doses), decreased body weight gain, increased serum glucose, increased kidney weights, cystic thyroid follicules, and an increased incidence of both benign and malignant hepatocellular liver tumors (males) were seen.

In a developmental toxicity study in rats there was evidence of increased qualitative susceptibility of fetuses to fluazinam; there was no evidence of increased quantitative susceptibility. Fetal exposure of 250 mg/kg/day resulted in decreases in body weights, decreased placental weights, and increased incidences of facial/palate clefts, diaphragmatic hernia and delayed ossification in several bone types. There was also greenish amniotic fluid and increases in late resorptions, as well as postimplantation loss. Maternal effects observed at the same dose level were decreases in body weight gain and food consumption, increases in water consumption, and increased urogenital staining.

There was no evidence of increased quantitative or qualitative susceptibility in a developmental toxicity study in rabbits or a 2-generation reproduction study in rats.
In the developmental rabbit study, there were decreases in food consumption and increased liver histopathology in maternal animals at 7 mg/kg/day. At the higher dose of 12 mg/kg/day, fetal toxicity was observed in the form of increased incidences of total litter resorptions and a slight increased incidence of fetal skeletal abnormalities (eg. kinked tail tip, fused or incompletely ossified sternebrae, and abnormalities of head bones). In the rat reproduction study, liver pathology (hepatocytic fatty changes) was observed in parental F_1 males at 9.7 mg/kg/day. Reproductive toxicity was manifested as a decreased number of implantation sites and decreased

litter sizes to day 4 post partum for F_1 females (F_2 litters) at 53.6 mg/kg/day. At 42.12 mg/kg/day, developmental effects observed were limited to decreased body weight gain during lactation for both F_1 and F_2 pups.

In an acute oral neurotoxicity study in rats, there were decreases in motor activity and soft stools observed on the day of dosing at 1000 mg/kg/day. These effects were considered due to systemic toxicity and not a result of frank neurotoxicity. In two subchronic neurotoxicity studies (evaluated together) in rats there were no signs of neurotoxicity observed up to 280 mg/kg/day. 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) 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 histopathology examination of several tissues of the CNS and occurred most frequently in the brain (sections of cerebrum and/or sections of cerebellum, pons, medulla, and midbrain) and less frequently in the cervical spinal cord. This lesion is reversible and is attributed to an impurity-5 (see section 3.3.2.).

In a combined chronic/carcinogenicity study in rats, increased incidences of thyroid gland follicular cell tumors were observed in male rats; there were no treatment-related increases in female rats. The Cancer Assessment Review Committee (CARC) concluded that there was some evidence that the thyroid tumors observed in the male rats were treatment-related. In two carcinogenicity studies in mice, increased incidences of hepatocellular tumors were observed in males with no treatment-related tumors observed in the females. In one study, the CARC concluded that there was clear evidence of treatment-related increases in both benign and malignant liver tumors in the male mice. In the other study, it was concluded that there was equivocal/some evidence for hepatocellular tumors in the male mice. There is no evidence of mutagenicity after exposure to fluazinam. In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 2, 1999), the CARC classified fluazinam into the category "Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential." The CARC also determined that the quantification of human cancer risk was not required.

3.1.3 Dose-response

For acute dietary exposure (females 13-49), the developmental toxicity study in rabbits was used to calculate the acute reference dose (aRfD) of 0.07 mg/kg/day. The developmental NOAEL of 7 mg/kg/day and the LOAEL of 12 mg/kg/day were based on increased incidences of total litter resorptions and slight increased incidences of fetal skeletal abnormalities. The aRfD of 0.5 mg/kg/day calculated for general population acute dietary exposure was based on a LOAEL of 1000mg/kg/day (NOAEL=50mg/kg/day) from an acute neurotoxicity study in rats; adverse effects seen were decreases in motor activity and soft stools on the day of dosing. For chronic dietary exposure (all populations), the carcinogenicity study in mice was used to calculate the chronic reference dose (cRfD) of 0.011 mg/kg/day). The NOAEL of 1.1 mg/kg/day and the LOAEL of 10.7 were based on adverse liver alterations (increased liver weights and histopathology). A 21-day dermal toxicity study in rats was used to select the dose and endpoint for occupational short- and intermediate-term dermal exposure. The NOAEL of 10 mg/kg/day and the LOAEL of 100 mg/kg/day were based on increased cholesterol and aspartate aminotransferase. For occupational short- and intermediate-term inhalation exposure, a 7-day

inhalation study in rats was used. The NOAEL of 1.38 and the LOAEL of 3.87 mg/kg/day were based on increased liver weights and testes weights in males. In the inhalation study, a histopathological examination was not performed; thus an additional factor of 10x was applied to the conventional uncertainty factor of 100x. This factor also addresses the use of a short-term (7 days) study to evaluate intermediate-term inhalation exposure. There are no residential uses proposed for fluazinam; therefore, incidental oral and residential dermal and inhalation risk assessments were not conducted.

3.2 Absorption, Distribution, Metabolism, Excretion (ADME)

Overall recovery of the administered radioactivity (reported in MRID Nos. 43521006, 43521007, and 43521008) was acceptable (93.10-103.55%). Excretion via the urine was minor. AMPA mercapturate and DAPA, the major urinary metabolites, represented only 0.05-0.39% of the administered dose. Radioactivity in the feces represented most of the administered dose (88.78-100.03%) as determined by review of MRID Nos. 43521006, 43521007, and 43521008. Identified fecal metabolites, however, represented from 11.20-68.59% of the administered dose. For all dose groups, most of the fecal radioactivity appeared to reside with unextractable components in the post-extraction solids (PES). Further analysis of the PES components using base hydrolysis indicated that most of this radioactivity could be attributed to hydrolysis products of AMPA and DAPA. PES radioactivity was also greatest for the low-dose group which was consistent with the lower overall accounting of identified metabolites for this group. Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the administered dose. With the exception of the low-dose group, parent compound represented most of the identified radioactivity in the feces. AMPA and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the administered dose (except for high-dose female rats where AMPA accounted for 10.22%).

DAPA glucuronide and AMPA mercapturate were the major biliary metabolites but represented < 4% of the administered dose. Total biliary radioactivity, however represented 25-34% of the administered dose (MRID Nos. 43521006, 43521007, and 43521008). Analysis of chromato-grams indicated that numerous other metabolites were present in the bile but were individually of insufficient quantity to allow for characterization.

Metabolite profiles from administration of different label positions (pyridyl and phenyl) indicated that there was no metabolic cleavage of the ring structures. Minor quantitative differences in metabolite recovery were observed between genders but not of sufficient magnitude to suggest biologically relevant differences in the metabolism of IKF-1216

3.3 FQPA Considerations

3.3.1 Adequacy of the Toxicity Database

3.3.2 Evidence of Neurotoxicity

There was no evidence of neurotoxicity observed in an acute neurotoxicity study (MRID 44807210) in rats up to 1000 mg/kg/day. Systemic effects observed were decreases in motor

activity and soft stools observed on the day of dosing. In two subchronic neurotoxicity studies (MRIDs 44807217& 44807218), evaluated together, there were no signs of neurotoxicity or systemic effects observed up to 280 mg/kg/day. 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) chronic studies in mice and dogs and later, upon careful re-examination of the CNS, also in shorter-term (4-week to 90-day) subchronic studies in mice and dogs. This lesion was observed during histopathological examination of several tissues of the CNS and occurred most frequently in the brain (sections of cerebrum and/or sections of cerebellum, pons, medulla, and midbrain) and less frequently in the cervical spinal cord. Although this lesion was also observed in control animals, the increased incidence and/or severity of the lesion in treated 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 among 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.

A developmental neurotoxicity study (MRID 46534401) was submitted to address the concerns regarding the white matter vacuolization observed in the subchronic and chronic studies in mice and dogs, as well as the increased qualitative susceptibility seen in the developmental rat study. In the study, there was no evidence of vacuolation of the brain or any other treatment-related pathology seen in dams or pups up to 50 mg/kg/day. Treatment-related effects observed in pups were decreases in body weight and body weight gain (lactation), and delayed preputial separation seen in the absence of maternal toxicity.

As previously stated, the brain lesions (vacuolation of the brain and nerve tissue) observed are attributed to impurity #5. The level of impurity 5 in the DNT study was 0.09%. At the current maximum concentration of Impurity-5 in technical grade fluazinam of 0.1% [see memorandum from Indira Gairola, Technical Review Branch, RD (7505C) to Cynthia Giles-Parker, Fungicide Branch, RD (7505C), dated May 18, 2001, DP Barcode D272455], the NOAEL for CNS effects

of 0.02 mg/kg/day for Impurity-5 is equivalent to a NOAEL for CNS effects of 20 mg/kg/day for technical grade fluazinam. The aRfDs (0.07 and 0.5 mg/kg/day) and cRfD (0.011 mg/kg/day), as well as risk assessments for dermal (10 mg/kg/day) and inhalation (1.38 mg/kg/day$_+$ additional 10x) exposures are set at doses much lower than 20 mg/kg/day. Therefore, they are protective of any possible neurotoxic effects resulting from exposure to impurity #5.

Based on the results of the developmental neurotoxicity study and the overall weight of the evidence, additional neurotoxicity studies are not needed.

3.3.3 Developmental Toxicity Studies

In a developmental toxicity study in rats, decreased body weight gain and food consumption and increased water consumption and urogenital staining were observed in maternal animals at the LOAEL of 250 mg/kg/day (NOAEL= 50 mg/kg/day). Additionally at 250 mg/kg/day, there were treatment-related decreases in body weights and placental weights; increases in facial/palate clefts, diaphragmatic hernia, and delayed ossification; as well as slight increases in late resorptions and postimplantation loss observed in fetuses. In the developmental toxicity study in rabbits, maternal effects included decreases in food consumption and liver histopathology at the LOAEL of 7 mg/kg/day (NOAEL=4 mg/kg/day). In fetuses, skeletal anomalies and an increased incidence of total litter resorptions were noted at 12 mg/kg/day (NOAEL=7 mg/kg/day).

3.3.4 Reproductive Toxicity Study

In a 2-generation reproduction study in rats, liver pathology (periacinar hepatocytic fatty changes) was observed in parental F_1 males at the LOAEL of 9.7 mg/kg/day (NOAEL=1.9mg/kg/day). Reproductive toxicity was seen in F_1 females (F_2 litters) at the LOAEL of 53.6 mg/kg/day (NOAEL=10.6 mg/kg/day) as a decreased number of implantation sites and decreased litter sizes up to day 4 post partum. At the LOAEL of 42.12 mg/kg/day (NOAEL=8.4 mg/kg/day), developmental effects observed were limited to decreased body weight gain during lactation for both F_1 and F_2 pups.

3.3.5 Additional Information from Literature Sources

A PubMed literature search indicated a publication that suggests that fluazinam may have immunotoxic potential. The reference is:

A. Draper et al (2003) Occupational asthma from fungicides fluazinam and chlorothalonil. Occupational and Environmental Medicine 60:76-77.

Fluazinam is a skin sensitizer; thus, it is a potential inhalation allergen with the potential to cause asthma. Although the cited study demonstrates that fluazinam causes asthmatic symptoms, the asthma effect may be related to the sensitization potential for this chemical. Consequently, asthmatic symptoms may only be detected in a small population of people who may develop hypersensitivity as a result of chronic exposure to small amounts of the chemical in an industrial setting. Therefore, the potential development of asthma in the general population is not anticipated and immunotoxicity tests are not required at this time. Additionally, current

immunotoxicity test guidelines focus on immunosuppression and are not designed to test for asthma, autoimmunity, or allergy (except dermal sensitization).

3.3.6 Pre-and/or Postnatal Toxicity

3.3.6.1 Determination of Susceptibility

There was evidence of increased qualitative susceptibility of fetuses to fluazinam observed in the developmental toxicity study in rats. In this study, increased incidences of facial/palate clefts and other rare deformities in the fetuses were observed in the presence of minimal maternal toxicity. A developmental neurotoxicity study in rats was submitted to address the increased susceptibility, as well as the presence of neurotoxic lesions observed after fluazinam exposure. In the developmental neurotoxicity study, decreases in body weight and body weight gain and a delay in completion of balano-preputial separation were observed in pups. These effects were seen in the absence of maternal effects, suggesting increased quantitative susceptibility of the offspring.

3.3.6.2 Degree of Concern Analysis and Residual Uncertainties for Pre- and/or Postnatal Susceptibility

The purposes of the Degree of Concern analysis are: (1) to determine the level of concern for the effects observed when considered in the context of all available toxicity data; and (2) to identify any residual uncertainties after establishing toxicity endpoints and traditional uncertainty factors to be used in the risk assessment. If residual uncertainties are identified, then HED determines whether these residual uncertainties can be addressed by a FQPA safety factor and, if so, the size of the factor needed.

Although there is qualitative evidence of increased susceptibility in young in the developmental toxicity study in rats, there are no residual uncertainties with regard to pre- and/or postnatal toxicity following *in utero* exposure to rats or rabbits and pre and/or post-natal exposures to rats. Considering the overall toxicity profile and the doses and endpoints selected for risk assessment for fluazinam, the degree of concern for the effects observed in the study is low. There is a clear NOAEL for the fetal effects seen, the effects occurred in the presence of maternal toxicity, and they were only seen at the highest dose tested. Additionally, the NOAEL of 50 mg/kg/day identified in this developmental toxicity study in rats is significantly higher than the NOAEL used (7 mg/kg/day) to establish the acute Reference Dose (aRfD) of 0.07 mg/kg/day (females 13-49); thus, the aRfD is protective of any potential developmental effects.

Increased quantitative evidence of susceptibility was observed in a developmental neurotoxicity study in rats (MRID 46534401). In pups, there were decreases in body weight and body weight gain during lactation, and delayed preputial separation observed at 10 mg/kg/day (NOAEL=2 mg/kg/day). Although the NOAEL of 2 mg/kg/day is lower than that used for the aRfD for females 13-49 (7 mg/kg/day), the effects noted in the developmental neurotoxicity study are attributable to multiple doses and are considered post-natal effects. Therefore, the study endpoint is not appropriate for acute dietary exposures. The cRfD of 0.011 mg/kg/day is based on a lower NOAEL of 1.1 mg/kg/day and is considered protective of potential developmental effects.

3.4 FQPA Safety Factor for Infants and Children

After evaluating the toxicological and exposure data, the fluazinam risk assessment team recommends that the FQPA SF be reduced to 1x based on the following:

The toxicological database for fluazinam is complete in regard to pre-and postnatal toxicity and neurotoxicity. There are acceptable developmental toxicity studies in rats and rabbits, and an acceptable reproduction study in rats; there is an acceptable developmental neurotoxicity study, as well as acute and subchronic neurotoxicity studies. In addition, there are a series of special studies investigating the neurotoxic lesions observed after fluazinam exposure.

The toxicity data for fluazinam showed increased qualitative susceptibility of fetuses to fluazinam in rats in a developmental toxicity study and neurotoxic lesions in studies in rats, mice and dogs. However, the developmental neurotoxicity study in rats and the special studies submitted adequately addressed the observed effects; thus, there are no residual uncertainties with regard to pre- and/or postnatal toxicity or neurotoxicity and no additional factors are needed (see 3.3.6.1 and 3.3.6.2).

The dietary food exposure assessment is based on HED-recommended tolerance- level residues and assumes 100% crop treated for all commodities, which results in very high-end estimates of dietary exposure. Actual exposures and risks from fluazinam will likely be lower.

The dietary drinking water assessment is based on values generated by model and associated modeling parameters which are designed to provide conservative, health protective, high-end estimates of water concentrations.

No residential uses are registered or proposed at this time.

3.5 Hazard Identification and Toxicity Endpoint Selection

3.5.1 Acute Reference Dose (aRfD) - Females age 13-49

Study Selected: Developmental toxicity study in rabbits
MRID No: 46578987
Dose and Endpoint for Risk Assessment: NOAEL= 7 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)

Comments about Study/Endpoint/Uncertainty Factors: A developmental toxicity study in rabbits was used to select the dose and endpoint for establishing the aRfD of 0.07 mg/kg/day. The NOAEL of 7 mg/kg/day and the LOAEL of 12 mg/kg/day were 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 sternebrae, and abnormalities of head bones). The skeletal abnormalities observed are considered effects that could occur after a single dose of fluazinam; thus, the route and duration of exposure are

appropriate for this population . Uncertainty factors (100x) include: 10x interspecies extrapolation and 10x intraspecies variability.

$$\textbf{Acute RfD (females 13-49)} = \frac{7\ mg\ /\ kg\ /\ day}{100\ (UF\ )} = \textbf{0.07 mg/kg/day}$$

3.5.2 Acute Reference Dose (aRfD) - General Population

Study Selected: Acute neurotoxicity study in rats
MRID No: 44807210
Dose and Endpoint for Risk Assessment: NOAEL= 50 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)

Study/Endpoint/Uncertainty Factors: An acute oral neurotoxicity study in rats was used to select the dose and endpoint for establishing the aRfD of 0.5 mg/kg/day. The NOAEL of 50 mg/kg/day and the LOAEL of 1000 mg/kg/day were based on soft stools and decreased motor activity. Due to the large dose spread in this study between the NOAEL (50 mg/kg/day) and the LOAEL (1000 mg/kg/day), the true NOAEL is probably much higher than 50 mg/kg/day. This study, however, provides the best data available for determining an acute RfD for the general population (including infants and children); the route and duration of exposure are appropriate for this population. Uncertainty factors (100x) include: 10x interspecies extrapolation and 10x intraspecies variability.

$$\textbf{Acute RfD (females 13-49)} = \frac{50\ mg\ /\ kg\ /\ day}{100\ (UF\ )} = \textbf{0.5 mg/kg/day}$$

3.5.3 Chronic Reference Dose (cRfD)

Studies Selected: A 2-Year carcinogenicity study in mice and a 1-Year chronic oral study in dogs (co-critical studies) were selected to establish the cRfD. The 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. The NOAELs in the mouse (1.12 mg/kg/day in males and 1.16 mg/kg/day in females) and the dog (1 mg/kg/day in males and females) studies and the LOAELs in the mouse (10.72 mg/kg/day in males and 11.72 mg/kg/day in females) and dog (10 mg/kg/day in males and females) studies were similar.

1st Study: 2-Year carcinogenicity study in mice
MRID No: 42208405, 44807220, 44807212
Dose and Endpoint for Risk Assessment: NOAEL= 1.12 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)

Study/Endpoint/Uncertainty Factors: A 2-Year carcinogenicity study in mice was one of two co-critical studies used in selecting the dose and endpoint for establishing the cRfD of 0.11 mg/kg/day. The NOAEL of 1.12 mg/kg/day and the LOAEL of 10.72 mg/kg/day were 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 route and duration of exposure are appropriate for this population. Uncertainty factors (100x) include: 10x interspecies extrapolation and 10x intraspecies variability.

2nd Study: 1-Year chronic oral toxicity study in dogs
MRID No: 42270603, 44807219
Dose and Endpoint for Risk Assessment: NOAEL= 1 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)
Study/Endpoint/Uncertainty Factors: A 1-Year chronic oral toxicity study in dogs was one of two co-critical studies used in selecting the dose and endpoint for establishing the cRfD of 0.11 mg/kg/day. The NOAEL of 1 mg/kg/day and the LOAEL of 10 mg/kg/day were based on marginal increases in the incidence of nasal dryness in females and the incidence/severity of gastric lymphoid hyperplasia in both sexes. The route and duration of exposure are appropriate for this population. Uncertainty factors (100x) include: 10x interspecies extrapolation and 10x intraspecies variability

$$\textbf{Chronic RfD} = \frac{1.1\ mg\ /\ kg\ /\ day}{100\ (UF\ )} = \textbf{0.011 mg/kg/day}$$

Comments: A 2-year chronic feeding/carcinogenicity study in rats (MRID 42248620, 44807223) was also considered for establishing the cRfD based on a lower NOAEL of 0.38 mg/kg/day. 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). A second 2-year chronic feeding study in rats (MRID 44839901, 44807213) was subsequently performed with 2 intermediate dose levels. The doses used in the study were 0, 1.0, 1.9, and 3.9 mg/kg/day for males; 0, 1.2, 2.4, and 4.9 mg/kg/day for females. 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. Therefore, this study was not chosen for risk assessment.

3.5.4 Dermal Absorption

A dermal absorption study is not available for fluazinam. A dermal absorption factor of 25% was estimated by comparing the LOAEL from a 21-day dermal toxicity study in rats (42270602) to the LOAEL from a 4-week range-finding feeding study in rats (44807213) based on a common endpoint (liver toxicity). In the dermal toxicity study, liver effects observed were increased aspartate aminotransferase (AST) and increased cholesterol levels in males at the LOAEL of 100 mg/kg/day (NOAEL=10 mg/kg/day). In the range-finding study, effects included increased serum phospholipids in females, increased total cholesterol in males and females, increased relative liver weights in females, liver histopathology (periacinar hypertrophy) in males, as well as decreased body weight gain and decreased food consumption in females at the LOAEL of 26.4 mg/kg/day in males (25.9 mg/kg/day in females) and the NOAEL of 5.1 mg/kg/day in males (5.3 mg/kg/day in females).

Estimated Dermal= Oral LOAEL x 100 = 25 mg/kg/day x 100 = **25%**
Absorption Factor Dermal LOAEL 100 mg/kg/day

3.5.5 Dermal Exposure (Short-, Intermediate-Term)

Study Selected: 21-Day dermal toxicity study in rats
MRID No: 42270602

Dose and Endpoint for Risk Assessment: NOAEL= 100 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)

Study/Endpoint/Uncertainty Factors: A 21-day dermal toxicity study in rats was used to select the dose and endpoint for short- and intermediate-term dermal exposure. The NOAEL of 10 mg/kg/day and the LOAEL of 100 mg/kg/day were based on increased AST and increased cholesterol levels in males. The route and duration of exposure are appropriate for this population. Uncertainty factors (100x) include: 10x interspecies extrapolation and 10x intraspecies variability.

Comments: Developmental effects were noted in developmental toxicity studies at ≥ 12 mg/kg/day; however, since the endpoint chosen for dermal risk assessments is based on a lower NOAEL (10 mg/kg/day) it is considered protective of potential developmental effects.

3.5.6 Inhalation Exposure (Short-, Intermediate-Term)

Study Selected: 7-Day range-finding inhalation study in rats (test material: Frowncide® WP, containing 51.9% fluazinam)
MRID No: 42248621
Dose and Endpoint for Risk Assessment: NOAEL= 1.38 mg/kg/day
Uncertainty Factor: 100x (10x interspecies extrapolation, 10x intraspecies variability)

Study/Endpoint/Uncertainty Factors: A 7-day range-finding inhalation study in rats was used to select the dose and endpoint for short- and intermediate-term intermediate exposure. The NOAEL of 0.011 mg/L (2.76 mg/kg/day in males and 2.97 mg/kg/day in females) and the LOAEL of 0.032 mg/L (7.93 mg/kg/day in males and 8.50 mg/kg/day in females) were based on slightly increased testes weights (males) and slightly increased liver weights (females). The test material used in the inhalation study was not technical grade fluazinam, but a formulation (Frowncide® WP) containing approximately 50% fluazinam. Consequently, the NOAEL from the study (0.011 mg/L or 2.76 mg/kg/day in males and 2.97 mg/kg/day in females) was 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); thus, for inhalation risk assessment a NOAEL of 1.38 mg/kg/day was used. A histopathology examination was not performed in the study and it is possible that the true NOAEL may be lower than that demonstrated in the study. An additional safety factor of 10x was applied to the conventional uncertainty factor of 100x to account for the lack of histopathology in the inhalation studies. This factor also addresses the use of a short-term (7 days) study to evaluate intermediate-term inhalation exposure. Uncertainty factors (1000x) include: 10x interspecies extrapolation, 10x intraspecies variability, and 10x for lack of histopathological examination and use of a short-term study for intermediate-term exposure. The

7-day range-finding inhalation study is the most appropriate route to use for evaluating inhalation risk. The target organ in the study is the liver, as seen in several other studies in the toxicology database.

Comments: Developmental effects were noted in developmental toxicity studies at ≥ 12 mg/kg/day; however, since the endpoint chosen for inhalation risk assessments is based on a lower NOAEL (1.38 mg/kg/day) it is considered protective of potential developmental effects.

Recommendations: Since the last risk assessment, an inhalation waiver request (subchronic inhalation study) has been submitted by the registrant for fluazinam. HED concludes that for the proposed new uses of fluazinam, a subchronic inhalation study is not required. However, HED recommends that a 28-day subchronic inhalation study be submitted to further characterize and support the registration of fluazinam. If an acceptable subchronic inhalation study is submitted and determined to be more appropriate for endpoint selection, the additional 10x safety factor can be reduced. HED reserves the right to require a subchronic inhalation study in the future for any proposed new uses.

3.5.7 Level of Concern for Margin of Exposure

Table 3.5.8. Summary of Levels of Concern for Risk Assessment.

Route	Short-Term (1 - 30 Days)	Intermediate-Term (1 - 6 Months)	Long-Term (> 6 Months)
Occupational (Worker) Exposure			
Dermal	100	100	N/A
Inhalation	1000	1000	N/A

3.5.8 Recommendation for Aggregate Exposure Risk Assessments

As per FQPA, 1996, when there are potential residential exposures to a pesticide, aggregate risk assessment must consider exposures from three major routes: oral, dermal, and inhalation exposures. As there are no registered or proposed residential uses an aggregate exposure (three routes) risk assessment is not required. Occupational dermal and inhalation exposures were not added since they were based on different endpoints.

3.5.9. Classification of Carcinogenic Potential

In accordance with *EPA's Draft Guidelines for Carcinogen Risk Assessment* (July, 1999), the Cancer Assessment Review Committee (CARC) classified (HED Doc. No.: 014512, March 29, 2001) fluazinam as "Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential", based on increases in thyroid gland follicular cell tumors in male rats and increases in hepatocellular tumors in male mice. The Agency has determined that quantification of human cancer risk is not required and the cRfD (0.011 mg/kg/day) is protective of potential cancer effects.

The cancer classification was based on the following weight-of-evidence considerations:

(i) There was some evidence that fluazinam induced an increase in thyroid gland follicular cell tumors in male rats, but not in female rats at ≥ 100 ppm (3.8 mg/kg/day). In one study in mice, there was clear evidence that an increased incidence of hepatocellular tumors observed in male mice was treatment related at 1000 ppm (107 mg/kg/day). In another study in mice, there was equivocal/some evidence that fluazinam may have induced an increase in hepatocellular tumors in the male mice at ≥ 3000 ppm (377mg/kg/day). Increases in hepatocellular tumors observed in the female mice in the latter study were not statistically significant and some occurred at an excessively toxic dose level. The thyroid gland follicular cell tumors of concern were seen only in the male rats and the hepatocellular tumors of concern were seen only in the male mice.

(ii) Fluazinam was negative in mutagenicity assays.

3.5.10 Summary of Toxicological Doses and Endpoints for Fluazinam for Use in Human Risk Assessments

Table 3.5a Toxicological Doses and Endpoints for Fluazinam for Use in Dietary and Non-Occupational Human Health Risk Assessments

Exposure/ Scenario	Point of Departure	Uncertainty/ FQPA Safety Factors	RfD, PAD, Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (General population)	NOAEL= 50 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF=1x Total UF=100x	Acute RfD =0.5 mg/kg/day aPAD = 0.5mg/kg/day	Acute Neurotoxicity-Rats. LOAEL = 1000 mg/kg/day based on decreased motor activity and soft stools on day of dosing.
Acute Dietary (Females 13-49 years of age)	NOAEL (developmental) = 7 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF=1x Total UF=100x	Acute RfD =0.07 mg/kg/day aPAD = 0.07mg/kg/day	Developmental Toxicity- Rabbits. Developmental LOAEL = 12 mg/kg/day based on increased incidence of total litter resorptions and possibly increased incidence of fetal skeletal abnormalities.
Chronic Dietary (All Populations)	NOAEL= 1.1 mg/kg/day	UF_A= 10x UF_H=10x FQPA SF=1x Total UF=100x	Chronic RfD =0.011 mg/kg/day cPAD = 0.011mg/kg/day	Carcinogenicity-Mice. LOAEL = 10.7 mg/kg/day based on liver histopathology and increased liver weight.
Cancer (oral, dermal, inhalation)	Classification: "Suggestive Evidence of Carcinogenicity, but not sufficient to assess human carcinogenic potential" .			

NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). FQPA SF = FQPA Safety Factor. PAD = population adjusted dose (a = acute, c = chronic). RfD = reference dose. N/A = not applicable.

Table 3.5b Summary of Toxicological Doses and Endpoints for Fluazinam for Use in Occupational Human Health Risk Assessments				
Exposure/ Scenario	**Point of Departure**	**Uncertainty Factors**	**Level of Concern for Risk Assessment**	**Study and Toxicological Effects**
Dermal Short-Term (1-30 days); Intermediate-Term (1-6 months)	NOAEL (systemic) 10 mg/kg/day	UF_A=10x UF_H=10x	Occupational LOC for MOE = 100	21-Day dermal, rats. Systemic LOAEL = 100 mg/kg/day based on increased cholesterol, increased aspartate aminotransferase.
Inhalation Short-Term (1-30 days)	Inhalation study NOAEL= 1.38 mg/kg/day	UF_A=10x UF_H=10x UF_{DB}=10x IAF=100%	Occupational LOC for MOE = 1000	7-Day inhalation, rats. LOAEL = 3.97 mg/kg/day based on increased liver weights (females) and increased testes weights.
Inhalation Intermediate-Term (1-6 months)	Inhalation study NOAEL= 1.38 mg/kg/day	UF_A=10x UF_H=10x UF_{DB}/UF_S =10x IAF=100%	Occupational LOC for MOE = 1000	7-Day inhalation, rats. LOAEL = 3.97 mg/kg/day based on increased liver weights (females) and increased testes weights.
Cancer (oral, dermal, inhalation)	Classification: "Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential"			

NOAEL = no observed adverse effect level. LOAEL = lowest observed adverse effect level. UF = uncertainty factor. UF_A = extrapolation from animal to human (interspecies). UF_H = potential variation in sensitivity among members of the human population (intraspecies). UF_S = use of a short-term study for long-term risk assessment. UF_{DB} = to account for the absence of key data (i.e., lack of a histopathological examination). MOE = margin of exposure. LOC = level of concern. IAF=inhalation absorption factor.

3.6 Endocrine disruption

EPA is required under the FFDCA, as amended by FQPA, to develop a screening program to determine whether certain substances (including all pesticide active and other ingredients) "may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effects as the Administrator may designate." Following recommendations of its Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), EPA determined that there was a scientific basis for including, as part of the program, the androgen and thyroid hormone systems, in addition to the estrogen hormone system. EPA also adopted EDSTAC's recommendation that the Program include evaluations of potential effects in wildlife. For pesticide chemicals, EPA will use FIFRA and, to the extent that effects in wildlife may help determine whether a substance may have an effect in humans, FFDCA authority to require the wildlife evaluations. As the science develops and resources allow, screening of additional hormone systems may be added to the Endocrine Disruptor Screening Program (EDSP).

When additional appropriate screening and/or testing protocols being considered under the Agency's EDSP have been developed, fluazinam may be subjected to further screening and/or testing to better characterize effects related to endocrine disruption.

4.0 Public Health and Pesticide Epidemiology Data

No public health/epidemiology data were used in developing this risk assessment.

5.0 Dietary Exposure/Risk Characterization

Acute and chronic aggregate dietary (food and drinking water) exposure and risk assessments were conducted using the Dietary Exposure Evaluation Model DEEM-FCID™, Version 2.03 which use food consumption data from the U.S. Department of Agriculture's Continuing Surveys of Food Intakes by Individuals (CSFII) from 1994-1996 and 1998. The analyses were conducted as part of a human health aggregate risk assessment for the requested uses of fluazinam on ginseng, *Brassica* vegetables, legume vegetables, and bushberries. An assessment of cancer risk is not necessary for this chemical since it is classified as "not likely to be carcinogenic to humans."

Both the acute and chronic analyses are based on tolerance-level residues, assume 100% crop treated, and incorporate modeled estimated drinking water concentrations (EDWCs). Therefore, the resulting exposure and risk estimates should be considered high-end and very conservative. Actual exposures and risks from fluazinam will likely be lower than the values presented in the analyses.

The acute risk estimates are below HED's level of concern for all population subgroups, including those of infants and children. Generally, HED is concerned when risk estimates exceed 100% of the population-adjusted dose (PAD). The acute risk estimate for the U.S. population, as a whole, is 1% of the acute PAD (aPAD). For females 13-49 years of age, the risk estimate is 8% of their aPAD. Risk estimates for all other population subgroups are less than 8% aPAD. Likewise, chronic risk estimates are below HED's level of concern for all population subgroups. The risk estimate for the U.S. population is 9% of the chronic PAD (cPAD). The highest risk estimate is for the All Infants (<1year) population subgroup at 16% cPAD (Table 4).

The analyses indicate that dietary exposure considerations do not preclude establishing the proposed tolerances for fluazinam.

5.1 Pesticide Metabolism and Environmental Degradation

5.1.1 Metabolism in Primary Crops

The nature of the residue in plants has been adequately delineated, based on acceptable potato, peanut, and grape metabolism studies reviewed previously (D257115; William Cutchin; 5/21/2001), along with an acceptable apple metabolism study submitted recently (MRID #46991301). The metabolism of fluazinam appears to be similar in potatoes, peanuts, grapes, and apples. Fluazinam undergoes reduction of one of the nitro groups to an amine, forming AMPA. AMPA may then be conjugated with glutathione, with subsequent degradation of the glutathione moiety to cysteine. The AMPA-cysteine conjugate then undergoes transamination, reduction, and conjugation with glucose to form AMGT. In addition, both rings of fluazinam appear to be labile to ring cleavage, and subsequent degradation of the rings into small fragments that may then be incorporated into a variety of natural plant components. HED concluded that

the ROC in potatoes and peanuts (for both tolerance expression and dietary risk assessment purposes) was the parent compound only (D272624; William Cutchin; 4/23/2001). In wine grapes, both parent and AMGT were included in the ROC for tolerance expression and risk assessment. Additionally, HED determined that data generated for potential new uses on other crops (with the exception of root and tuber, and bulb vegetables) should include analyses for both parent and AMGT.

5.1.2 Metabolism in Rotational Crops

Regulatory requirements pertaining to fluazinam residues in rotational crops have been fulfilled, and the rotational crop restrictions on the proposed label are adequate. An adequate confined rotational crop study was previously reviewed (D212612, D216941, and D217467; George Herndon; 9/5/1995) in conjunction with a time-limited tolerance petition for use on peanuts. The available data indicate that neither fluazinam nor any of its structurally related metabolites are likely to be detectable in rotational root crops, and leafy vegetables planted ≥30 days (≥68 days for small grains, and all other crops) following a 2.0 lb ai/A (1X) soil application of fluazinam.

5.1.3 Metabolism in Livestock

The nature of the residue in livestock is also understood, based on adequate goat and hen metabolism studies (D257115; William Cutchin; 5/21/2001). The metabolism of [^{14}C]-fluazinam in ruminants and poultry is similar, and involves reduction of one or both nitro groups on the phenyl ring to form AMPA, MAPA, or DAPA. Fluazinam also undergoes dehalogenation and hydroxylation of the chlorine on the phenyl ring to form HYPA. These compounds may then undergo conjugation with glutathione, and subsequent degradation of the glutathione component yields a variety of polar compounds. Although the ring structure of the parent molecule remains intact, fluazinam *per se* was only a minor component (≤2.7% TRR) of the [^{14}C]-residues in poultry tissues and eggs, and was not detected in ruminant tissues or milk. The fluazinam residues of regulatory interest in animals were determined by HED to be parent plus the metabolites AMPA and DAPA, and their sulfamate conjugates.

5.1.4 Analytical Methodology

Fluazinam: The tolerance-enforcement method, *Fluazinam: Method for the Analysis in Peanut Nut Meat* (MRID #43521016), was adequately radiovalidated. This GC/ECD method for determining residues of fluazinam *per se* was originally reviewed in conjunction with the time-limited tolerance petition for peanuts (D177127 and D177137; George Herndon; 6/19/1992). In brief, residues of fluazinam are extracted from crop samples with MeOH/acetic acid (HOAc) (50:1, v/v), filtered, acidified with 0.2N HCl, and partitioned into hexane. Residues are then partitioned into 0.5N NaOH, the aqueous phase is acidified, and residues are partitioned back into hexane. The resulting hexane fraction is concentrated, and residues are purified using a Florisil column, then analyzed by GC/ECD. The petitioner achieved adequate recoveries of fluazinam from peanut nutmeat samples fortified with fluazinam at 0.010-1.00 ppm. This method has undergone a successful ILV trial (D212612, D216941, and D217467; George Herndon; 9/5/1995) using peanut nutmeats fortified with fluazinam at 0.010, 0.020, and 0.050 ppm. Recoveries at the 0.010 ppm level were low (56% and 68%) owing to an interference peak; therefore, the validated LOQ would be 0.020 ppm. However, the independent laboratory

noted that the method could possibly be improved in the Florisil clean-up step. The method was forwarded to ACB for a PMV trial, and was subsequently determined to be suitable as a tolerance-enforcement method (D266802; Paul Golden; 6/22/2001).

The submitted GC/ECD methods (modifications of the tolerance-enforcement method) are adequate for collecting data and tolerances enforcement on residues of fluazinam *per se* in the various crop commodities associated with this petition. The LLMV and/or LOQ for residues of fluazinam *per se* were 0.010 ppm in all plant matrices except snap beans and lima beans, in which the LLMV and LOQ were 0.020 ppm.

AMGT: The submitted HPLC/UV method (a working method based on *Method Evaluation for the Analysis of AMGT in Grapes*, MRID #45593101) is adequate for collecting data on AMGT residues in blueberries. Blueberries were blended with acetonitrile (ACN)/water (4:1, v:v), and filtered. The filter paper with contents was extracted a second time. The combined solvent extract was then concentrated by evaporation. The sample was partitioned with 2% aqueous Na_2SO_4 and methylene chloride. The aqueous layer was acidified to a pH of <1 with 6N HCl, then partitioned twice with EtOAc, and the organic phase was evaporated to dryness. The aqueous sample was applied to a C_{18} SPE column, and AMGT was eluted with ACN/water (3:7; v:v). After evaporation to dryness, the sample was taken up in ACN/H_2O/HOAc, and filtered through a 0.45 µm PTFE disc prior to analysis by HPLC/UV at 256 nm. The LLMV, LOD, and LOQ were 0.020, 0.013, and 0.038 ppm, respectively, for residues of AMGT in blueberries. HED has previously determined that residues of AMGT are to be regulated in wine grapes (D272624; William Cutchin; 4/23/2001). The Agency therefore requested that this method undergo an ILV trial, and, potentially, a PMV trial by the ACB. An ILV study has not yet been submitted.

As there are currently no tolerances established in livestock commodities, and none are needed as a result of the requested uses, residue analytical methods for livestock commodities are not required.

5.1.5 Environmental Degradation

Based on the properties of the chemical, applications of fluazinam are likely to reach the target (the crop), but drift is also possible. The chemical has a low vapor pressure, and a moderate Henry's Law constant. Due to the fact that it appears to show relatively short half lives in aquatic media, and it binds to soils, EFED believes that the chemical would not volatilize substantially.

EFED concludes that fluazinam appears to degrade at moderate to low rates in aerobic soils, but it is more rapidly transformed into other compounds of similar backbone structure in high pH solutions or in aquatic media, both, aerobic or anaerobic. Fluazinam may be photolyzed relatively rapidly (2.5 days) to form a tricyclic compound (G-504). The total fluazinam residues, fluazinam and its transformation products (DCPA, CAPA, and DAPA) are persistent in most environments (aerobic aquatic metabolism 51-71 days, relatively stable in anaerobic aquatic environment) and are likely to reach aquatic media as a totality through runoff. Since fluazinam does not alter substantially its backbone structure in the environment, but instead, goes through a slight transformation of functional groups, EFED considered parent and transformation products

together when making assessments.

While the parent and two transformation products, HYPA and CAPA, have relatively low mobility, indicating a relatively low potential for ground water contamination, further information on the other transformation products should be required in a new terrestrial field dissipation study.

Fluazinam shows a potential to bioaccumulate in fish (BCF=1220X for whole fish; ≥67% of residues depurated in 21 days).

The fate and transport characterization also summarizes the various degradation products formed by each process in the studies reviewed in tabular form. (Table 5.1.5)

Table 5.1.5. Summary of degradate formation from degradation of fluazinam.

STUDY TYPE	DEGRADATE and MAXIMUM CONCENTRATION			SOURCE
	CAPA (% applied)	HYPA (% applied)	AMPA (% applied)	
Hydrolysis	34% at 28 days pH 7; 84-85% at 20 days at pH 9	–	–	MRID: 42208412.
Aqueous Photolysis	G-504 was 14.0-17.1% by 7-10 days	–	–	MRID: 44807312, 43521009.
Soil Photolysis	–	Detected at more than dark control	Detected at more than dark control	MRID: 44807313.
Aerobic Soil Metabolism	–	Detected	MAPA and DAPA also detected	MRID: 42208413.
Aerobic Aquatic Metabolism	–	–	24.2% at 0.2 day; DAPA: at day 30; SDS-67200 39.6%by day 14	MRID: 43521010.
Anaerobic Aquatic Metabolism	12.6% at 72 hr	–	DAPA: 19.0% by 240 hr; DCPA: 11.3% at 24 hr	MRID: 44807314.
Terrestrial Field Dissipation	MAPA, CAPA, and HYPA were monitored; however, there were problems with the storage stability data			MRID: various.

5.1.6 Comparative Metabolic Profile

Data depicting the metabolism of fluazinam in plants and animals, as well as data on environmental degradates, have been submitted to the Agency.

In rats, fluazinam metabolism involves hydroxylation followed by conjugation. The major urinary metabolites identified were AMPA mercapturate and DAPA at 0.05-0.39% of administered dose (AD). Radioactivity in the feces represented most of the AD (88.78-100.03%); however, metabolites identified represented only 11.20-68.59% the AD. Fecal

radioactivity (all dose groups) appeared to reside with unextractable components in the post-extraction solids (PES), which upon further analysis was attributed to hydrolysis products of AMPA and DAPA. Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the AD. With the exception of the low-dose group (greatest PES radioactivity), parent compound represented most of the identified radioactivity in the feces. AMPA and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the AD (except for high-dose female rats where AMPA accounted for 10.22%). DAPA glucuronide and AMPA mercapturate were the major biliary metabolites (<4% of the AD).

The metabolism of [^{14}C]-fluazinam in ruminants and poultry is similar, and involves reduction of one or both nitro groups on the phenyl ring to form AMPA, MAPA, or DAPA. Fluazinam also undergoes dehalogenation and hydroxylation of the chlorine on the phenyl ring to form HYPA. These compounds may then undergo conjugation with glutathione, and subsequent degradation of the glutathione component yields a variety of polar compounds. Although the ring structure of the parent molecule remains intact, fluazinam *per se* was only a minor component (≤2.7% TRR) of the [^{14}C]-residues in poultry tissues and eggs, and was not detected in ruminant tissues or milk

In plants, fluazinam undergoes reduction of one of the nitro groups to an amine, forming AMPA. AMPA may then be conjugated with glutathione, with subsequent degradation of the glutathione moiety to cysteine. The AMPA-cysteine conjugate then undergoes transamination, reduction, and conjugation with glucose to form AMGT. In addition, both rings of fluazinam appear to be labile to ring cleavage, and subsequent degradation of the rings into small fragments that may then be incorporated into a variety of natural plant components.

5.1.7 Toxicity Profile of Major Metabolites and Degradates of Concern

There is no toxicology information available on fluazinam metabolites and degradates**.**

5.1.8 Pesticide Metabolites and Degradates of Concern

Table 5.1.8 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression

Matrix		**Residues included in Risk Assessment**	**Residues included in Tolerance Expression**
Plants	Primary Crop: grapes	Parent fluazinam and AMGT	Parent fluazinam and AMGT
	Primary Crop: peanuts, root/tuber vegetables	Parent fluazinam	Parent fluazinam
	Primary Crop: all others	Parent fluazinam and AMGT	Parent fluazinam
	Rotational Crop	N/A	**Note:** Tolerances not required based on the absence of residues in rotational crops at the requested plant back interval.

Table 5.1.8 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression			
Matrix		**Residues included in Risk Assessment**	**Residues included in Tolerance Expression**
Livestock	Ruminant	Parent fluazinam, AMPA, and DAPA, and their sulfate conjugates	Parent fluazinam, AMPA, and DAPA, and their sulfate conjugates
	Poultry	Parent fluazinam, AMPA, and DAPA, and their sulfate conjugates	Parent fluazinam, AMPA, and DAPA, and their sulfate conjugates
Drinking Water		Parent Fluazinam, CAPA, DAPA, DCPA	Not Applicable

At a meeting held on 11/28/2000, HED concluded that the residue of concern (ROC) in potatoes and peanuts (for both tolerance expression and dietary risk assessment purposes) was the parent compound only (D272624; William Cutchin; 4/23/2001). In wine grapes, both parent and AMGT were included in the ROC for tolerance expression and risk assessment. Additionally, HED determined that data generated for potential new uses on other crops (with the exception of root and tuber, and bulb vegetables) should include analyses for both parent and AMGT.

The fluazinam residues of regulatory interest in animals were determined by HED to be parent plus the metabolites AMPA and DAPA, and their sulfamate conjugates.

Estimated drinking water concentrations (EDWC's) were calculated for Total Fluazinam Residues along with EDWC's for parent fluazinam since the environmental fate studies indicated that the parent compound forms transformation compounds (CAPA, HYPA, and AMPA) which are similar in structure to the parent (under most conditions). Given that HED is unable to conclude all these degradates are significantly less toxic than the parent, the drinking water assessment is based on total residues of fluazinam and its major degradates.

5.1.9 Drinking Water Residue Profile

The drinking water residue used in the dietary risk assessment was provided by the Environmental Fate and Effects Division (EFED; J. Meléndez, D334948, 7 Feb 2007) and incorporated directly into this dietary assessment into the food categories "water, direct, all sources" and "water, indirect, all sources." The estimated drinking water concentration (EDWC) of 0.071 ppm is the estimated peak concentration of fluazinam parent or total residues from the FIRST model (for more information, see http://www.epa.gov/oppefed1/models/water/) and was used for the acute assessment. The chronic assessment uses the EDWC of 0.0177 ppm based on total residues of fluazinam in surface water from the FIRST model (Table 5.1.9).

Table 5.1.9. Maximum Tier I Estimated Drinking Water Concentrations (EDWCs) for drinking water assessment based on ground application of fluazinam.			
Drinking Water Source (Model Used)	**USE (Rate Modeled)**	**Maximum Estimated Drinking Water Concentration (EDWC; ppb)**	
Groundwater (SCI-GROW) Fluazinam and Total Residues of Fluazinam	Bushberries (3.90 lb a.i./A)	Acute and Chronic	0.187
Surface Water (FIRST) Fluazinam	Bushberries (3.90 lb a.i./A)	Acute	71.0
	Bushberries (3.90 lb a.i./A)	Chronic	0.7
Surface Water (FIRST) Total Residues of Fluazinam	Bushberries (3.90 lb a.i./A)	Acute	71.0
	Bushberries (3.90 lb a.i./A)	Chronic	17.7

5.1.10 Food Residue Profile

Residue chemistry issues relevant to the proposed new uses requested in the current petitions were reviewed in the *Summary of Analytical Chemistry and Residue Data* memorandum for fluazinam (D335640; W. Drew).

Adequate storage stability data were collected indicating that fluazinam residues were stable under frozen storage in blueberries, snap beans, and broccoli for the storage durations and conditions of the samples from the respective crop field trials. In blueberries, AMGT residues were stable under frozen storage for the storage durations and conditions of the samples from the blueberry field trials. However, storage stability studies indicated that there was significant dissipation of fluazinam residues under frozen storage in ginseng, lima beans, dried beans, cabbage, and mustard greens. Correction factors were therefore incorporated into the recommended tolerances for fluazinam in ginseng, shelled succulent beans, and shelled dried beans to account for dissipation during storage. A correction factor was not utilized when setting the recommended tolerance in *Brassica* leafy vegetables, because fluazinam applications made to cabbage and mustard greens were essentially identical to the treatment of broccoli (which had acceptable storage stability), and all residues in treated samples from the *Brassica* field trials were ≤LOQ (≤0.010 ppm). At the time of submission, the freezer storage stability analyses were not completed for the AAFC cabbage field trial. A final report is expected shortly. Pending submission of the final report for AAFC Project AAFC03-066R, the storage stability data generated for IR-4 Project 08796 are adequate to support the storage conditions and durations of the cabbage samples from the AAFC field trial.

There are currently no tolerances for fluazinam established in livestock commodities, and there are no significant livestock feed items associated with the proposed uses.

Regulatory requirements pertaining to fluazinam residues in rotational crops have been fulfilled, and the rotational crop restrictions on the proposed label are adequate.

The crop field trial data are adequate, and support the proposed use patterns. Adequate numbers of trials were conducted in the appropriate geographical regions, and samples were analyzed for the ROC using adequate methods. However, residue data for AMGT were provided only for blueberries; AMGT data should also have been included with the field trial studies for edible-podded beans, shelled succulent and dried beans, and *Brassica* vegetables. Residues of fluazinam in treated blueberry samples ranged from 0.064 to 2.0 ppm, and residues of AMGT

ranged from 0.025 to 0.13 ppm (with combined residues of 0.166-2.094 ppm) at the target PHI of 30 days (23-32 days). The maximum residue observed in snap beans treated with a single application of fluazinam, and harvested at PHIs of 14-28 days, was 0.029 ppm, detected in a single sample. All remaining samples had residues below the LOQ (<0.020 ppm). The maximum residue observed in snap beans treated with two applications of fluazinam, and harvested at PHIs of 10-22 days, was 0.109 ppm. No quantifiable (<LOQ; <0.010 ppm) or detectable (<LOD; <0.003 ppm) residues of fluazinam were reported in any broccoli sample harvested 50 to 113 days after a single root-drench application of fluazinam at the time of transplant. No quantifiable (<LOQ; <0.010 ppm) or detectable (<LOD; <0.005 ppm) residues of fluazinam were reported in any cabbage sample harvested 58 to 104 days after a single root-drench application of fluazinam at the time of transplant. No residues above the LLMV (the maximum residue observed was 0.010 ppm) were reported in any mustard greens sample harvested 22 to 78 days after a single root-drench application of fluazinam at the time of transplant. In the trials performed at the 1X and 2X application rates, the residues of fluazinam in ginseng ranged from 0.28 to 1.4 ppm, and 2.1 to 2.2 ppm, respectively. The storage stability study, however, raises the possibility that actual residues in ginseng (at harvest) were up to 50% greater than the quantitated results, based on in-storage dissipation of fluazinam. Fluazinam residues were less than the LLMV (<0.010 ppm) in all dried bean samples from the field trials, except for one sample at 0.0114 ppm. The storage stability study, however, raises the possibility that actual residues in dried beans (at harvest) were up to 50% greater than the quantitated results, based on in-storage dissipation of fluazinam. Fluazinam residues were less than the LOQ (<0.020 ppm) in all lima bean samples from the field trials. The storage stability study, however, raises the possibility that actual residues in lima beans (at harvest) were up to 50% greater than the quantitated results, based on in-storage dissipation of fluazinam.

There are no processed commodities for which residue data are required associated with the proposed uses on the crops requested in the subject petitions under review.

Table 5.1.10. Summary of New-Use Food Crop Residue Levels Used in the Acute and Chronic Dietary Exposure Analyses.

Crop/Crop Group	Recommended Tolerance Level, ppm	Residue Level for Dietary Exposure Assessment, ppm[a]
Ginseng	4.5	4.5[b]
Brassica Vegetables (Group 5)	0.01	0.0135
Edible Podded Legumes (except peas; Group 6A)	0.1	0.135
Succulent Shelled Pea and Bean (Group 6B)	0.04	0.054
Dried Shelled Pea and Bean (Group 6C)	0.02	0.027
Bushberries (Group 13B)	*Not needed	*Not needed

[a] Residue level = recommended tolerance × 1.35 (from grape metabolism data)
[b] AMGT is not a significant residue in root and tuber crops and no correction is necessary for risk assessment
[c] Residue level = recommended tolerance × 1.11 (from field trial data)
* Tolerance not needed, low bush blueberry is a member of the bushberries subgroup 13-B

5.1.11 International Residue Limits

There are no established or proposed Canadian or Codex MRLs for residues of fluazinam in plant or animal commodities. There are Mexican MRLs established for residues of fluazinam in potato at 0.05 ppm, and in beans at 0.1 ppm. The recommended US tolerance of 0.10 ppm in edible-podded beans will be in harmony with the existing 0.1 mg/kg MRL for Mexico.

Tolerances on shelled beans are being established at lower levels than the Mexican MRL based on residues reflecting the use proposed in the U.S.

5.2 Dietary Exposure and Risk

Fluazinam acute and chronic dietary exposure assessments were conducted using the Dietary Exposure Evaluation Model software with the Food Commodity Intake Database DEEM-FCID™,Version 2.03 which incorporates consumption data from USDA's Continuing Surveys of Food Intakes by Individuals (CSFII), 1994-1996 and 1998. Both the acute and chronic assessments are based on tolerance-level residues, with worst-case assumptions regarding levels of the metabolite AMGT. In addition, it was assumed that all crops with registered or proposed uses of fluazinam were treated (*i.e.*, 100% crop treated). These assumptions result in highly conservative, health-protective estimates of exposure and risk.

5.2.1 Acute Dietary Exposure/Risk

The acute risk estimates are below HED's level of concern for all population subgroups, including those of infants and children. Generally, HED is concerned when risk estimates exceed 100% of the population-adjusted dose (PAD). The acute risk estimate for the U.S. population, as a whole, is 1% of the acute PAD (aPAD). For females 13-49 years of age, the risk estimate is 8% of the aPAD. Risk estimates for all other population subgroups are less than 8% of the aPAD.

5.2.2 Chronic Dietary Exposure/Risk

The chronic risk estimates are also below HED's level of concern for all population subgroups. The risk estimate for the U.S. population is 9% of the chronic PAD (cPAD). The highest risk estimate is for the All Infants (<1year) population subgroup at 16% cPAD.

5.2.3 Cancer Dietary Risk

Fluazinam is classified as demonstrating "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential." The chronic dietary exposure analysis is considered to be protective of cancer effects. As a result, a separate cancer risk assessment was not performed.

Table 5.2. Summary of Acute and Chronic Exposure and Risk Estimates for Fluazinam.

Population Subgroup	Acute Assessment (95th Percentile)			Chronic Assessment		
	PAD, mg/day	Exposure Estimate, mg/day	% Pad	PAD, mg/day	Exposure Estimate, mg/day	% Pad
U.S. Population	0.5	0.006015	1	0.011	0.000953	9
All infants	0.5	0.015211	3	0.011	0.001799	16
Children 1-2 yrs	0.5	0.007019	1	0.011	0.001133	10
Children 3-5 yrs	0.5	0.006323	1	0.011	0.000996	9
Children 6-12 yrs	0.5	0.004439	1	0.011	0.000650	6
Youth 13-19 yrs	0.5	0.003344	1	0.011	0.000438	4
Adults 20-49 yrs	0.5	0.005903	1	0.011	0.000996	9
Adults 50+ yrs	0.5	0.006933	1	0.011	0.001123	10
Females 13-49 yrs	0.07	0.005809	8	0.011	0.001016	9

5.3 Anticipated Residue and Percent Crop Treated (%CT) Information

The acute and chronic analyses assumed tolerance level residues, 100% crop treated, and DEEM™ (ver. 7.81) default processing factors for all registered and proposed commodities. For those processed commodities in the DEEM-FCID™ residue list which were not in DEEM™ (ver 7.81) (e.g., flour, bran, etc.), a processing factor of 1 was assumed.

6.0 Residential (Non-Occupational) Exposure/Risk Characterization

Currently, there are no registered or proposed residential uses for fluazinam; thus, there is no exposure via this pathway and an assessment was not conducted.

Spray Drift

Based on the proposed label restrictions, DO NOT apply this product in a way that will contact workers or other persons, either directly or through drift. Aerial application of this product is prohibited.

Spray drift is a potential source of exposure for residents living in close proximity to spraying operations. This situation is particularly the case with aerial application. However, to a lesser extent, spray drift resulting from the ground application of fluazinam could also be a potential source of exposure. The Agency has been working with the Spray Drift Task Force (a membership of US pesticide registrants), EPA Regional Offices, State Lead Agencies for pesticide regulation, and other parties to develop the best spray drift management practices. The Agency is now requiring interim mitigation measures for aerial applications that must be placed on product labels/labeling. The Agency has completed its evaluation of the new database submitted by the Spray Drift Task Force, and is developing a policy on how to appropriately apply the data and the AgDRIFT computer model to its risk assessments for pesticides applied by air, orchard airblast, and ground hydraulic methods. After the policy is in place, the Agency may impose further refinements in spray drift management practices to reduce off-target drift, and risks associated with pesticide application.

7.0 Aggregate Risk Assessments and Risk Characterization

7.1 Acute Aggregate Risk

Acute aggregate risk consists of risks resulting from exposure to residues in food and drinking water alone. The acute dietary exposure analysis included both food and drinking water. As a result, the acute aggregate risk assessment is equivalent to the acute dietary risk assessment and risk estimates are below HED's level of concern.

7.2 Short-Term Aggregate Risk

As there are no residential uses for fluazinam, short-term aggregate risk assessments were not conducted.

7.3 Intermediate-Term Aggregate Risk

As there are no residential uses for fluazinam, intermediate-term aggregate risk assessments were not conducted.

7.4 Long-Term Aggregate Risk

The chronic aggregate risk assessment consists of risks resulting from exposure to residues in food, drinking water, and residues resulting from residential applications. As there are no residential uses for fluazinam, chronic aggregate risk consists of risks resulting from exposure to residues in food and drinking water alone. The chronic dietary exposure analysis included both food and drinking water. As a result, the chronic aggregate risk assessment is equivalent to the chronic dietary risk assessment and risk estimates are below HED's level of concern.

7.5 Cancer Risk

Fluazinam was classified as demonstrating "suggestive evidence of carcinogenicity, but not sufficient to assess carcinogenic potential." The chronic aggregate assessment is considered to be protective of cancer effects.

8.0 Cumulative Risk Characterization/Assessment

Section 408(b)(2)(D)(v) of the FFDCA requires that, when considering whether to establish, modify, or revoke a tolerance, the Agency consider "available information concerning the cumulative effects" of a particular pesticide's residues and "other substances that have a common mechanism of toxicity."

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to fluazinam and any other substances, and fluazinam does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, EPA has not assumed that fluazinam has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

9.0 Occupational Exposure/Risk Pathway

9.1 Agricultural Handler Risk

The following products have been assessed for occupational exposure: OMEGA® 500F and Allegro® 500F. The products are formulated as a flowable suspension/liquid containing 40.0 % fluazinam. It may be applied by airblast or groundboom at application rates ranging from 0.45-1.36 lbs active ingredient (ai) per acre (A) for a single application and rates from 0.91-3.9 lbs ai per acre per growing season. Based on the anticipated application practices for the OMEGA® 500F and Allegro® 500F Fungicide, product labels and information provided by the registrant,

handler exposures are expected to be short- and intermediate-term in duration. The quantitative risk assessment developed for handlers is based on the following exposure scenarios:

- Mixing/Loading Liquid formulation for groundboom
- Mixing/Loading Liquid formulation for airblast
- Applying sprays using groundboom
- Applying sprays using airblast

No chemical-specific data for assessing exposure during pesticide handling activities were submitted to the Agency in support of this Section 3 application. Therefore, HED used data from the Pesticide Handlers Exposure Database (PHED) Version 1.1 to assess handler exposures for regulatory actions when chemical-specific data are not available (HED Science Advisory Council for Exposure, SOP Number .007, January 1999).

9.1.2 Handlers Exposure and Risk

HED's level of concern (LOC) for occupational dermal exposures is 100 (i.e., MOE less than 100 is of concern). The level of concern for inhalation exposures is 1000 (i.e., MOE less than 1000 is of concern). All dermal risk estimates for short- and intermediate-term handler exposure resulted in MOEs greater than 100 with the use of gloves. All inhalation risk estimates for short- and intermediate-term handler exposure resulted in MOEs greater than 1000 with the use of a dust mist respirator (Table 9.1.2.).

As reflected in the calculations included in Table 9.1.2, PPE consisted of the addition of chemical-resistant gloves and a dust/mist respirator to the baseline attire in order for all scenarios to reach either dermal MOEs of 100 or inhalation MOEs of 1000. However, since fluazinam is classified as a Toxicity Category I chemical for acute eye irritation, HED recommends that the PPE requirements (i.e. chemical-resistant gloves, chemical resistant footwear, coveralls, protective eyewear, dust/mist respirator and chemical resistant apron when mixing and loading) on the proposed label are followed by all handlers for acute/local toxicity to reduce systemic exposure.

Table 9.1.2 Short- and Intermediate-term Handler Exposure and Risk for Fluazinam

Exposure Scenario (Scenario #)	Mitigation Level	Dermal Unit Exposure (mg/lb ai)	Inhalation Unit Exposure (µg/lb ai)	Crop	Single Application Rate	Amount Treated	Dermal Dose (mg/kg/day)	Dermal MOE	Inhalation Dose (mg/kg/day)	Inhalation MOE
Mixing/Loading										
Liquid for Groundboom Application (PHED)	Single Layer, No Gloves	2.9	1.2 (0.0012 mg/lb ai)	Dry Bean and Succulent Bean Crop (Subgroup 6B except Peas); Edible-podded Legume Vegetables (Subgroup 6A except Peas	0.45 lb ai/A	80 A	1.49	7	0.00062	2,200
				Ginseng	0.78 lb ai/A		2.59	4	0.00107	1,300
				Brassica (Cole) Leafy Vegetables (Group 5)	1.36 lb ai/A		4.51	2	0.00187	740
	Single Layer, Gloves	0.023		Dry Bean and Succulent Bean Crop (Subgroup 6B except Peas); Edible-podded Legume Vegetables (Subgroup 6A except Peas	0.45 lb ai/A		0.012	830	0.00062	2,200
				Ginseng	0.78 lb ai/A		0.021	480	0.00107	1,300
				Brassica (Cole) Leafy Vegetables (Group 5)	1.36 lb ai/A		0.036	280	0.00186	740
	Single Layer, Gloves; Dust/Mist Respirator		0.00024 mg/lb ai	Brassica (Cole) Leafy Vegetables (Group 5)	1.36 lb ai/A		0.036	280	0.00037	3,700

Exposure Scenario (Scenario #)	Mitigation Level	Dermal Unit Exposure (mg/lb ai)	Inhalation Unit Exposure (μg/lb ai)	Crop	Single Application Rate	Amount Treated	Dermal Dose (mg/kg/day)	Dermal MOE	Inhalation Dose (mg/kg/day)	Inhalation MOE
Liquid for Airblast Application (PHED)	Single Layer, No Gloves	2.9	1.2 (0.0012 mg/lb ai)	Bushberry	0.65	40 A	1.07	10	0.00044	3,100
	Single Layer, Gloves	0.023					0.009	1,100	0.00044	3,100
Applicator										
Sprays for Groundboom Application (PHED)	Single Layer, Gloves	0.014	0.74 (0.00074 mg/lb ai)	Dry Bean and Succulent Bean Crop (Subgroup 6B except Peas); Edible-podded Legume Vegetables (Subgroup 6A except Peas)	0.45 lb ai/A	80 A	0.0072	1,400	0.00038	3,600
				Ginseng	0.78 lb ai/A		0.0124	810	0.00066	2,100
				Brassica (Cole) Leafy Vegetables (Group 5)	1.36 lb ai/A		0.0218	460	0.00115	1,200
Sprays for Airblast Application (PHED)	Single Layer, No Gloves	0.36	4.5 (0.0045 mg/lb ai)	Bushberry	0.65 lb ai/A	40 A	0.134	70	0.00167	830
	Single Layer, Gloves; Dust/Mist Respirator	0.24	0.0009 mg/lb ai				0.089	110	0.00033	4,200

Dermal and Inhalation Unit Exposures = PHED Version 1.1
Amount Treated = HED's Exposure Science Advisory Committee SOP Number 9.1,
Short- and Intermediate-term Dermal (mg/kg/day) = [Rate (lb ai/A) x UE (mg /lb ai) x DAF (100%) x Amount Treated (A/day)] / BW (70 kg)
Short- and Intermediate-term Dermal MOE = [Dermal NOAEL (10 mg/kg/day)] / Dermal Dose (mg/kg/day)
Short- and Intermediate-term Inhalation Dose (mg/kg/day) = [Rate (lb ai/A) x UE (mg /lb ai) x Amount Treated (A/day)] / BW (70 kg)
Short- and Intermediate-term Inhalation MOE = [Inhalation NOAEL (1.38 mg/kg/day)] / Inhalation Dose (mg/kg/day)

9.2 Postapplication Risk

9.2.1 Data and Assumptions for Postapplication Exposure Scenarios

Two chemical-specific postapplication studies were submitted in support of this registration action:

- "*Foliar Dissipation of Fluazinam from Potato Leaves Treated with Omega® 500F – USA in 2004*" (MRID# 469913-03); Report dated February 6, 2006. Author: Author: J.L. Wiedmann; Sponsor: Ishihara Sangyo Kaisha, Ltd; Performing Laboratories: EN-CAS Analytical Laboratories and

- "*Foliar Dissipation of Fluazinam from Peanut Leaves Treated with Omega® 500F – USA in 2004*" (MRID# 469913-02); Report dated May 22, 2006. Author: J.L. Wiedmann; Sponsor: Ishihara Sangyo Kaisha, Ltd; Performing Laboratories: EN-CAS Analytical Laboratories

These studies have been reviewed using the U.S. Environmental Protection Agency's (U.S. EPA) OPPT Series 875, Occupational and Residential Exposure Test Guidelines, Group B: Dislodgeable Foliar Residue Dissipation: Agricultural, Guideline 875.2100. These studies were designed to determine the dissipation of foliar residues (FR) of fluazinam and its metabolites in and on potato foliage and peanuts. The residue component of those studies has been extracted for chemical-specific use in determining the dislodgeable foliar residue (DFR) values for each sampling interval (Table 9.2.1a).

Table 9.2.1a: Summary of Peanuts and Potatoes Dissipation Foliar Residues Data

Location	Application Rate (lb ai/A)	Application Method	R-Squared	Slope (Ln DFR vs. t)	$T_{½}$ (days)	Time after application	Maximum value of Total Fluazinam Residue ($\mu g/cm^2$)	Coefficient of Variation (%)
Potatoes (MRID# 469913-03)								
Fayette County, Ohio	0.45	Backpack Sprayer	0.958	-0.21342	3.25 (Application #4)	Immediately after sprays dried	**3.01**	6.98
						0.33 day	2.91	5.08
						1 day	2.71	19.1
						5 days	0.58	16.9
						8 days	0.351	18.6
						13 days	0.125	17.4
						22 days	0.034	32.2
Peanuts (MRID# 469913-02)								
Sampson County, North Carolina	0.78	Tractor Mounted Sprayers	0.914	-0.07569	9.16 (Application #3)	Immediately after sprays dried	**1.11**	10.5
						1 day	1.51	8.86
						4 days	0.814	8.8
						7 days	0.623	14.1
						14 days	0.282	16.5
						21 days	0.202	17.7
						27 days	0.147	33
						35 days	0.107	27.8

9.2.2 Postapplication Exposure and Risk

Chemical-specific postapplication data were submitted in support of this registration action. For purposes of comparison, a Tier 1 (HED standard assumptions and defaults of 20% DFR) and Tier 2 (dislodgeable foliar residue data) analysis were performed to ensure that potential postapplication exposures are not of concern. A comparison of Tier 1 and Tier 2 analyses resulted in similar postapplication exposure risks of concern (MOE< 100). Tables 9.2.2a and b provide a summary of postapplication exposure and risk resulting from Tier 1 and Tier 2 analysis.

Since the Tier 1 and Tier 2 analyses resulted in similar postapplication exposure risks of concern, HED based its postapplication assessment on the Tier 2 analysis. The only crop scenarios which resulted in MOEs greater than 100 on day 0 (immediately after application) were for low exposure activities ((i.e., scouting, hand weeding, thinning and irrigation) for beans and ginseng. All other crops (i.e. bushberries, brassica and leafy vegetables) did not reach a MOE greater than or equal to 100 for low exposure activities until 3 to 13 days later. All medium (i.e., scouting, hand weeding and irrigation) and high (i.e., hand harvesting/ pruning/pinching/training) postapplication exposure activities for all crops resulted in MOEs below 100 on day of application. Crops did not reach MOEs greater than or equal to 100 until 4 to 20 days later depending on the specific crop.

Since postapplication exposure resulting in MOEs below 100 may indicate possible risk for re-entry of workers, HED provided a comparison of the estimated number of days required before a MOE of 100 is reached (i.e. restricted entry interval – REI) based on Tier 2 analysis to establish pre-harvest interval (PHI). **HED recommends that the Registration Division ensure that the PHIs do not go below the calculated REIs for harvesting**. Table 9.2.2c provides a summary of the REIs and PHIs for each crop and activity.

A comparison of Tier 1 and 2 analyses resulted in similar postapplication exposure risks of concern (MOE< 100). The tier 2 analyses resulted in slightly refined exposure risks by reducing the number of days at which a MOE of 100 was achieved. However, in a few instances the Tier 2 approach resulted in an increase in the number of days required before reaching that MOE. The 2 postapplication estimates of exposure may be overestimating residues on the proposed crops based on the methodology used to determine dislodgeable residues. However, HED cannot refine these estimates without chemical specific data collected in accordance with Agency guideline methods. A possible option for the registrant would be to repeat the DFR studies using guideline methods (i.e., leaf punch and dislodgeable residues with surfactant as opposed to whole leaf extraction).

Fluazinam has a low volatility, with a vapor pressure of 1.7 x 10-7 mmHg (2.3 x 10-5 Pa). Short-term postapplication inhalation exposures are expected to be minimal and less than the application exposures since negligible fluazinam vapor is expected to volatilize from the field. Consequently, a quantitative postapplication inhalation exposure assessment was not performed.

Restricted Entry Interval

The technical material has an Acute Eye Irritation Toxicity Category I. Per the Worker Protection Standard (WPS), a 48-hr restricted entry interval (REI) is required for chemicals

classified under Toxicity Category I. The 48 hour REI appearing on the label is only appropriate for postapplication activities for which the MOE reaches 100 on day 0. However, note that an interval of 3 to 20 days is necessary to reach a MOE of 100 for medium and high postapplication exposure activities (i.e., hand weeding/harvesting/pruning/pinching/training, and irrigation). **HED recommends that the proposed label be revised to ensure that the appropriate REI restrictions are clearly stated for all crops and do not exceed the pre-harvest intervals.**

Table 9.2.2a: Short- and Intermediate Term Postapplication Exposure and Risk for Fluazinam Using HED Default of 20% Dislodgeable Residue

Crops	Application Rate (lb ai/A)	Transfer Coefficients [a]	DAT[b]	DFR[c] (ug/cm^2)	Daily Dose[d] (mg/kg/day)	MOE[e]
Dry Bean and Succulent Bean Crop Edible-podded Legume Vegetables	0.45	Low (100)	0	1.01	0.012	870
		Med (1,500)	5	0.595	0.102	100
Bushberry (Low and High bush)	0.65	Low (400) (Low bush)	0	1.45	0.067	150
		High (1,500) (Low bush)	9	0.564	0.097	100
		Low (500) (High bush)	0	1.456	0.083	120
		High (1,100) (High bush)	6	0.774	0.097	100
Ginseng	0.78	Low (100)	0	1.75	0.02	500
		Med (1,500)	10	0.609	0.104	96
Brassica (Cole)	1.36	Low (2,000)	18	0.457	0.105	96
		High (5,000)	27	0.177	0.101	99
Leafy Vegetables		Low (500)	5	1.79	0.103	97
		High (2500)	20	0.370	0.106	94

a. Transfer Coefficient = estimated dermal transfer coefficients from the Science Advisory Council For Exposure Policy Number 3.1: Agricultural Transfer Coefficients, August 2000
b. DAT = Days After Treatment
c. DFR = Dislodgeable Foliar Residue (For Tier I) = Application Rate (lb ai/A) x (1- Daily Dissipation Rate) ^{t} x $4.54E^8$ ug/lb x $2.47E^{-8}$ A/cm^2 x 0.2
d. Daily Dose = [DFR (ug/cm^2) x TC (cm^2/hr) x 0.001 mg/ug x DAF (100%) x 8 hrs/day] ÷ Body Weight (70 kg),
e. MOE = NOAEL/Daily Dose (NOAEL = 10 mg/kg/day)

Table 9.2.2b: Short- and Intermediate Term Postapplication Exposure and Risk for Fluazinam Using DFR Studies

Crops	Study	Application Rate (lb ai/A)	Transfer Coefficients	DAT[a]	DFR[b] (ug/cm^2)	Adjusted DFR[c] (ug/cm^2)	Daily Dose[d] (mg/kg/day)	$MOE^{e}_{rounded}$
Dry Bean, Succulent Bean Crop , Edible-podded Legume Vegetables	Peanuts data MRID# 46991302	0.45	Low (100)	0	1.110	0.666	0.0076	1300
			Med (1,500)	4	0.814	0.4884	0.084	120
Bushberry (Low and High bush)	Potatoes data MIRD# 46991303	0.65	Low (400) (Low bush)	3	1.645 *	2.3	0.105	95
			High (1,500) (Low bush)	7	0.428 *	0.59	0.10	100
			Low (500) (High bush)	4	1.1125 *	1.557	0.0889	110
			High (1,100) (High bush)	5	0.58	0.812	0.105	100
Ginseng		0.78	Low (100)	0	3.010	5.220	0.059	170
			Med (1,500)	8	0.351	0.607	0.104	100
Brassica (Cole)		1.36	Low (2,000)	13	0.125	0.380	0.0863	120
			High (5,000)	20	0.054 *	0.163	0.093	110
Leafy Vegetables			Low (500)	5	0.58	1.75	0.10	100
			High (2500)	14	0.115*	0.348	0.099	100

a. DAT = Days After Treatment;
b. DFR = Dislodgeable Foliar Residue from the submitted studies;
b.* Back calculations for DFR values corresponding to days not listed on Table 9.2.1a are provided in D340845 (Z. Figueroa).
c. Adjusted DFR = DFRs were adjusted to compensate for difference in application rates (AR) between study and actual label application rates = (AR label proposed $rate_r$/AR study rate) x $DFR_{study;}$
d. Daily Dose = [DFR (ug/cm^2) x TC (cm^2/hr) x 0.001 mg/ug x DAF (100%) x 8 hrs/day] ÷ Body Weight (70 kg),
e. MOE = NOAEL (10 mg/kg/day) /Daily Dose (mg/kg/day)

Table 9.2.2c: Comparison of Crop REIs to PHIs

Crops	Transfer Coefficients	DAT REI	MOE	PHI
Dry Bean and Succulent Bean Crop	Med (1,500)	4	120	30
Eddible podded legumes				14
Bushberry (Low bush)	Low (400)	3	95	30
	High (1,500)	7	100	
Bushberry (High bush)	Low (500)	4	110	
	High (1,100)	5	100	
Ginseng	Med (1,500)	8	100	30
Brassica (Cole)	Low (2,000)	13	120	50
	High (5,000)	20	110	
Leafy Vegetables	Low (500)	5	100	20
	High (2500)	14	100	

DAT = Days After Treatment required to reach MOE > 100 = REI

10.0 Data Needs and Label Recommendations

10.1 Toxicology

A 28-day subchronic inhalation study is recommended to support the registration of fluazinam. If an acceptable subchronic inhalation study is submitted and determined to be more appropriate for endpoint selection, the additional 10x safety factor can be reduced.

10.2 Residue Chemistry

No major deficiencies were noted in the subject petition that would preclude the establishment of permanent tolerances for fluazinam residues in the requested crops. Revised Sections F should be submitted, so that the proposed tolerances reflect the recommended tolerance levels, and correct commodity definitions, as specified in Table C1 (Appendix C). Issues pertaining to residue chemistry deficiencies should be resolved (see below).

> As a condition of registration, results of an ILV trial for the AMGT analytical method (with wine grapes) should be submitted by the registrant. If the registrant agrees with the modifications made by Ricerca to the original method (in MRID #45593101), these modifications should be incorporated into a revised method for the ILV. Sample sets should include, at the minimum, 2 control (untreated) samples of wine grapes, 2 samples fortified at the tolerance level (3.0 ppm), and 2 samples fortified at the LOQ (0.010 ppm).
>
> As a condition of registration, MRM recovery data should be provided for the metabolite AMGT, since it is included in the tolerance expression for wine grapes.
>
> The product label for Omega 500F should be amended to include a restriction, stating that turnip roots from turnip plants treated with this EP must not be used for human nor livestock consumption.
>
> The Agency has previously determined, and the registrant is hereby advised again, that residue data for AMGT should be provided in the crop field trial studies for all future requested plant commodities, except root and tuber, and bulb vegetables.

10.3 Occupational and Residential Exposure

HED recommends that the Registration Division ensure that the PHIs do not go below calculated REIs for harvesting. Additionally, HED recommends that the proposed label be revised to ensure that the appropriate REI restrictions are clearly stated for all crops and correspond to the postapplication activities and reentry intervals.

References:

Dietary and Residue Chemistry

Fluazinam. Tolerance Petitions Requesting the Establishment of Permanent Tolerances (Associated with Section 3 Registration) for Food Use of the Herbicide on Edible-Podded Beans (Subgroup 6-A, Except Peas), Shelled Succulent Beans (Subgroup 6-B, Except Peas), Shelled Dried Beans (Subgroup 6-C, Except Peas), *Brassica* (Cole) Vegetables (Group 5), Bushberries (Subgroup 13-B), and Ginseng. Summary of Analytical Chemistry and Residue Data. W. Drew. D335640. W. Drew. 2007.

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Occupational and Residential Exposure

Fluazinam: Occupational Exposure/Risk Assessment for the Use on Ginseng, Dry Beans, Edible-podded Legume Vegetables, Bushberry and *Brassica* Leafy Vegetables. D340845. Z. Figueroa. 2007

Foliar Dissipation of Fluazinam from Potato Leaves Treated with Omega® 500F – USA in 2004" (MRID# 469913-03); Report dated February 6, 2006. Author: Author: J.L. Wiedmann;

Sponsor: Ishihara Sangyo Kaisha, Ltd; Performing Laboratories: EN-CAS Analytical Laboratories.

Foliar Dissipation of Fluazinam from Peanut Leaves Treated with Omega® 500F – USA in 2004" (MRID# 469913-02); Report dated May 22, 2006. Author: J.L. Wiedmann; Sponsor: Ishihara Sangyo Kaisha, Ltd; Performing Laboratories: EN-CAS Analytical Laboratories

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Fluazinam-2nd Report of the Hazard Identification Committee. TXR 0051576. Data Package Submitted by E. Budd. February, 19, 2003.

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Fluazinam: PP # 9F05079. EPA File Symbol 71512-R. New Reduced Risk Active Ingredient. Toxicology Disciplinary Chapter for the Registration Support Document and Data Evaluation Records (DERs) for All Recently Submitted Toxicology Studies, Toxicology Studies Not Previously Reviewed, and Previously Reviewed Toxicology Studies for Which Amended DERs or Updated Executive Summaries Have Been Prepared. D258235. E. Budd. June 18, 2001.

Memorandum from Indira Gairola, Technical Review Branch, RD (7505C) to Cynthia Giles-Parker, Fungicide Branch, RD (7505C). DP Barcode D272455. May 18, 2001.

Appendix A: Toxicology Assessment

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for a food use for fluazinam are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Test	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity	yes	yes
870.1200 Acute Dermal Toxicity	yes	yes
870.1300 Acute Inhalation Toxicity	yes	yes
870.2400 Primary Eye Irritation	yes	yes
870.2500 Primary Dermal Irritation	yes	yes
870.2600 Dermal Sensitization	yes	yes
870.3100 Oral Subchronic (rodent)	yes	yes
870.3150 Oral Subchronic (nonrodent)	yes	yes
870.3200 21/28-Day Dermal	yes	no
870.3250 90-Day Dermal	no	---
870.3465 90-Day Inhalation	no	---
870.3700a Developmental Toxicity (rodent)	yes	yes
870.3700b Developmental Toxicity (nonrodent)	yes	yes
870.3800 Reproduction	yes	yes
870.4100a Chronic Toxicity (rodent)	yes	yes
870.4100b Chronic Toxicity (nonrodent)	yes	yes
870.4200a Oncogenicity (rat)	yes	yes
870.4200b Oncogenicity (mouse)	yes	yes
870.4300 Chronic/Oncogenicity	yes	yes
870.5100 Mutagenicity—Gene Mutation - bacterial	yes	yes
870.5300 Mutagenicity—Gene Mutation - mammalian	yes	yes
870.5375 Mutagenicity—Structural Chromosomal Aberrations	yes	yes
870.5395 Mutagenicity—Other Genotoxic Effects	yes	yes
870.6100a Acute Delayed Neurotox. (hen)	no	---
870.6100b 90-Day Neurotoxicity (hen)	no	---
870.6200a Acute Neurotox. Screening Battery (rat)	yes	yes
870.6200b Chronic Neurotox. Screening Battery (rat)	yes	yes
870.6300 Develop. Neuro	yes	yes
870.7485 General Metabolism	yes	yes
870.7600 Dermal Penetration	no	---

A.2 Toxicity Profiles

Table 1. Acute Toxicity Data on Fluazinam Technical

Guideline No./ Study Type	Test Substance	MRID No.	Results	Toxicity Category
870.1100 Acute oral toxicity rats	Technical grade fluazinam (lot #109; 95.3%)	42248603	M: LD50 = 4500 mg/kg F: LD50 = 4100 mg/kg	III
	Technical grade fluazinam (lot #8412-20; 95.3%)	42248602	M: LD50 >5000 mg/kg F: LD50 >5000 mg/kg	IV
	Technical grade fluazinam (lot #1/87; 97.9%)	42248604	M: LD50 >5000 mg/kg F: LD50 >5000 mg/kg	IV
870.1200 Acute dermal toxicity rats	Technical grade fluazinam (lot #8303-2; 98.5%)	42248605	M: LD50 >2000 mg/kg F: LD50 >2000 mg/kg	III
870.1300 Acute inhalation toxicity rats	Technical grade fluazinam (lot #109; 95.3%)	42270601	M: LC50 = 0.463 mg/L F: LD50 = 0.476 mg/L	II
870.2400 Acute eye irritation rabbits	Technical grade fluazinam (lot # SNPE B-1216, No. 1006; 97.9%)	42248606	Extremely irritating. Corneal opacity did NOT reverse in 21 days.	I
870.2500 Acute dermal irritation rabbits	Technical grade fluazinam (lot # SNPE B-1216, No. 1006; 97.9%)	42248607	Slightly irritating	IV
870.2600 Dermal sensitization guinea pigs	Technical grade fluazinam (lot # 1030/91; 96.7%)	42274401	Positive	NA
	Ultra-purified fluazinam (lot #Y910401; 100%)	42248608	Negative	NA

Table 2. Acute Toxicity Data on Fluazinam (formulation)				
Guideline No./ Study Type	**Test Substance**	**MRID No.**	**Results**	**Toxicity Category**
870.1100 Acute oral toxicity rats	Omega 500F (40% fluazinam)	42974907	M: LD_{50} >5000 mg/kg F: LD_{50} >5000 mg/kg	IV
870.1200 Acute dermal toxicity rabbits	Omega 500F (40% fluazinam)	42974908	M: LD_{50} >2000 mg/kg F: LD_{50} >2000 mg/kg	III
870.1300 Acute inhalation toxicity rats	Fluazinam 50% WP [(1)] (51.3% fluazinam)	42311001	M: LC_{50} = 3.0 mg/L F: LD_{50} = 3.4 mg/L	IV
870.2400 Acute eye irritation rabbits	Omega 500F (40% fluazinam)	42974910	Slightly irritating	III
870.2500 Acute dermal irritation rabbits	Omega 500F (40% fluazinam)	42974911	Moderately irritating	II
870.2600 Dermal sensitization guinea pigs	Omega 500F (40% fluazinam)	42974912	Positive	NA

[(1)] Study satisfies requirement for testing on Omega 500F.

Table 3. Subchronic, Chronic and Other Toxicity Table		
Guideline No. Study Type	**MRID No./ (year) Classification /Doses**	**Results**
870.3100 90-Day oral toxicity rats	42248610 (1984); 44807214 (1998) Acceptable/guideline M : 0, 0.15, 0.77, 3.8, 38 mg/kg/day; F: 0, 0.17, 0.86, 4.3, 44 mg/kg/day	NOAEL: Males: 3.8 mg/kg/day; Females: 4.3 mg/kg/day LOAEL Males = 38 mg/kg/day; Females = 44 mg/kg/day based on increased liver weights and liver histopathology in males, and increased lung and uterus weights in females.
870.3150 90-Day oral toxicity dogs	42248611 (1991); 44807215 (1998) Acceptable/guideline M & F: 0, 1, 10, 100 mg/kg/day	NOAEL = 10 mg/kg/day LOAEL = 100 mg/kg/day based on retinal effects, increased relative liver weight, liver histopathology and possible increased serum alkaline phosphatase in females and possible marginal vacuolation of the cerebral white matter (equivocal)
870.3200 21-Day dermal toxicity rats	42270602 (1985) Acceptable/guideline M & F: 0, 10, 100, 1000 mg/kg/day	**Systemic** NOAEL = 10 mg/kg/day LOAEL = 100 mg/kg/day based on increased AST and cholesterol levels in clinical chemistry determinations (males) **Dermal** NOAEL = not identified LOAEL = 10 mg/kg/day based on erythema, acanthosis, and dermatitis

Table 3. Subchronic, Chronic and Other Toxicity Table		
Guideline No. Study Type	**MRID No./ (year) Classification /Doses**	**Results**
870.3250 90-Day dermal toxicity	NA	NA
870.3465 90-Day inhalation toxicity	NA	NA
870.3700a Prenatal developmental toxicity rats	42248613 (1985) Acceptable/guideline F: 0,10, 50, 250 mg/kg/day	**Maternal** NOAEL = 50 mg/kg/day LOAEL = 250 mg/kg/day based on decreased body weight gain and food consumption and increased water consumption and urogenital staining **Developmental** NOAEL = 50 mg/kg/day LOAEL = 250 mg/kg/day based on decreased fetal body weights and placental weights, increased facial/cleft palates, diaphragmatic hernia, and delayed ossification in several bone types, greenish amniotic fluid and possible increased late resorptions and postimplantation loss
870.3700b Prenatal developmental toxicity rabbits	42248616 (1988) Acceptable/guideline F: 0, 2, 4, 7, 12 mg/kg/day	**Maternal** NOAEL = 4 mg/kg/day LOAEL = 7 mg/kg/day based on decreased food consumption and increased liver histopathology. **Developmental** NOAEL = 7 mg/kg/day LOAEL = 12 mg/kg/day based on an increase in total litter resorptions and possible fetal skeletal abnormalities
870.3700b Prenatal developmental toxicity rabbits	42248615 (1985); 42248614 (1984); 42248617 (1984) Unacceptable/guideline F: 0, 0.3, 1, 3 mg/kg/day	**Maternal** NOAEL = 3 mg/kg/day LOAEL = not identified (>3 mg/kg/day) **Developmental** NOAEL = 3 mg/kg/day LOAEL = not identified (>3 mg/kg/day)
870.3800 Reproduction and fertility effects rats	42248619 (1987); 42208406 (1985); 42248618 (1986) Acceptable/guideline F_0 males: 0, 1.5, 7.3, 36.6 mg/kg/day F_0 females: 0, 1.7, 8.4, 42.1 mg/kg/day F_1 males: 0, 1.9, 9.7, 47.3 mg/kg/day F_1 females: 0, 2.2, 10.6, 53.6 mg/kg/day	**Parental/Systemic** NOAEL = 1.9 mg/kg/day LOAEL = 9.7 mg/kg/day based on liver pathology in F_1 males **Reproductive** NOAEL = 10.6 mg/kg/day LOAEL = 53.6 mg/kg/day based on decreased number of implantation sites and decreased litter sizes to day 4 post-partum for F_1 females (F_2 litters). **Offspring** NOAEL = 8.4 mg/kg/day LOAEL = 42.1 mg/kg/day based on reduced F_1 and F_2 pup body weight gains during lactation.
870.4100a Chronic toxicity rats	44839901 (1993) Acceptable/guideline M: 0, 1.0, 1.9, 3.9 mg/kg/day F: 0, 1.2, 2.4, 4.9 mg/kg/day	NOAEL = Males: 1.9 mg/kg/day; Females: 4.9 mg/kg/day LOAEL = Males: 3.9 mg/kg/day; Females: not identified (>4.9 mg/kg/day) based on increased testicular atrophy in males and no effects in females
870.4100b Chronic toxicity	42270603 (1987); 44807219 (1998) Acceptable/guideline	NOAEL = 1 mg/kg/day LOAEL = 10 mg/kg/day based on gastric lymphoid

Table 3. Subchronic, Chronic and Other Toxicity Table		
Guideline No. Study Type	**MRID No./ (year) Classification /Doses**	**Results**
dogs	M & F: 0, 1, 10, 50 mg/kg/day	hyperplasia in both sexes and nasal dryness in females
870.4300 Combined chronic toxicity/carcino-genicity rats	42248620 (1988); 44807223 (1999); 45150201 (2000) Acceptable/guideline M: 0, 0.04, 0.38, 3.8, 40 mg/kg/day F: 0, 0.05, 0.47, 4.9, 53 mg/kg/day	NOAEL = Males: 0.38 mg/kg/day; Females: 0.47 mg/kg/day LOAEL = Males: 3.8 mg/kg/day; Females: 4.9 mg/kg/day based on liver toxicity in both sexes, pancreatic exocrine atrophy in females and testicular atrophy in males. **Some evidence of carcinogenicity (thyroid gland follicular cell tumors) in male rats, but not in females**.
870.4200b Carcinogenicity mice	42208405 (1988); 4807220 (1996) Acceptable/guideline M: 0, 0.12, 1.1, 10.7, 107 mg/kg/day F: 0, 0.11, 1.2, 11.7, 117 mg/kg/day	NOAEL = Males:1.1 mg/kg/day; Females: 1.2 mg/kg/day LOAEL = Males: 10.7 mg/kg/day; Females: 11.7 mg/kg/day based on increased incidences of brown macrophages in the liver of both sexes, eosinophilic vacuolated hepatocytes in males, and increased liver weight in females **Clear evidence of carcinogenicity (hepatocellular tumors) in male mice, but not in females**
870.4200b Carcinogenicity mice	44807222 (1996); 44807221 (1998); 45201301 (2000) Acceptable/guideline M: 0, 126, 377, 964 mg/kg/day F: 0, 162, 453, 1185 mg/kg/day	NOAEL = Males:<126 mg/kg/day, Females: <162 mg/kg/day LOAEL = Males: 126 mg/kg/day; Females: 162 mg/kg/day based on increased liver weights and liver and brain histopathology in both sexes **Equivocal/some evidence of carcinogenicity (hepatocellular tumors) in male mice, but not in females**
870.5100 Bacterial reverse mutation assay (Ames test)	42270605 (1988) Acceptable/Guideline Up to 2 µg/plate for *S. typhimurium* strains and up to 250 µg/plate for *E. coli* (-S9). Up to 100 µg/plate for *S. typhimurium* strains and up to 500 µg/plate for *E. coli* (+ S9)	Negative with and without S9 up to cytotoxic concentrations.
870.5100 Bacterial reverse mutation assay (Ames test)	42270604 (1989) Acceptable/Guideline Up to 1µg/plate for *S. typhimurium* strains and up to 250 µg/plate for *E. coli* (-S9) and up to 100 µg/plate for *S. typhimurium* strains and up to 500 µg/plate for *E. coli* (+S9)	Negative with and without S9 up to cytotoxic concentrations.
870.5300 *In vitro* mammalian gene mutation assay	45261801 (2000) Acceptable/guideline Up to 9 µg/ml (+S9), up to	Negative with S9 activation up to 9 µg/ml. Negative without S9 activation up to 0.3 µg/ml. Compound tested to cytotoxic concentrations.

Table 3. Subchronic, Chronic and Other Toxicity Table		
Guideline No. Study Type	**MRID No./ (year) Classification /Doses**	**Results**
	0.3 µg/ml (-S9)	
870.5300 *In vitro* mammalian gene mutation assay	45156902 (1986) Acceptable/guideline Up to 5 µg/ml (+/-S9)	Negative with and without S9 activation up to 5 µg/ml. Compound tested to cytotoxic concentrations.
870.5375 *In vitro* mammalian chromosome aberration (CHL cells)	42270606 (1988) Acceptable/guideline Up to 9.5 µg/ml (+S9) up to 4 µg/ml (-S9)	Negative with and without S9 up to cytotoxic concentrations. Cells harvested at 24 and 48 hours in nonactivated studies and at 24 hours in activated studies.
870.5395 Mammalian erythrocyte micronucleus test	44807224 (1999) Acceptable/guideline 500, 1000, 2000 mg/kg (oral gavage)	Negative at 24 hour sacrifice (500, 1000, 2000 mg/kg). Negative at 24, 48, and 72 hour sacrifices (2000 mg/kg).
870.5550 UDS in primary rat hepatocytes	45156901 (1984) Unacceptable/guideline 0.05 to 6.25 µg/ml	Negative; however there were several serious study deficiencies: treatment time shorter than recommended, no data supporting the claim of cytotoxicity, data variability for major endpoints.
870.5550 Differential killing/growth inhibition in *B. subtilis*	42270607 (1988) Unacceptable/guideline 0.003 to 0.3 µg/disk (-S9) 0.3 to 30 µg/disk (+S9)	Negative, however only one replicate plate/dose was used.
870.6200a Acute neurotoxicity screening battery rats	44807210 (1995) Acceptable/guideline M & F: 0, 50, 1000, 2000 mg/kg	**Systemic** NOAEL = 50 mg/kg LOAEL = 1000 mg/kg based on soft stools and decreased motor activity on day of dosing. **Neurotoxicity** NOAEL = 2000 mg/kg LOAEL = not identified (>2000 mg/kg)
870.6200b Subchronic neurotoxicity screening battery rats	44807217 (1998); 44807218 (1998) Acceptable/guideline M :0, 21, 69, 74, 149, 233 mg/kg/day; F: 0, 23, 81, 89, 175, 280 mg/kg/day	Neurotoxicity NOAEL = Males: 233 mg/kg/day; Females: 280 mg/kg/day LOAEL = not identified (Males: >233 mg/kg/day; Females: > 280 mg/kg/day)
870.6300 Developmental neurotoxicity	46534401, 47018301 and 47037001. Acceptable/Non-Guideline 0, 2, 10 or 50 mg/kg/day	Maternal: NOAEL not established. No toxicity at highest dose tested. Developmental: NOAEL = 2 mg/kg/day. LOAEL = 10 mg/kg/day based on decreased pup weight and gain and delayed balano-preputial separation.

Table 3. Subchronic, Chronic and Other Toxicity Table		
Guideline No. Study Type	**MRID No./ (year) Classification /Doses**	**Results**
870.7485 Metabolism and pharmacokinetics rats	44807233 (1995); 43521004 thru 43521008, 43553001 (1993-1995) Acceptable/guideline 0.5, 50 mg/kg	Only 33-40% of the administered dose was absorbed. Most of the administered dose was recovered in the feces (>89%). Excretion via the urine was minor (<4%). Total biliary radioactivity, however, represented 25-34% of the administered dose, indicating considerable enterohepatic circulation.

A.3 Executive Summaries

A.3.1 Subchronic Toxicity

870.3100 90-Day Oral Toxicity – Rat

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 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). The NOAEL in this study is 50 ppm (3.8 mg/kg/day in males and 4.3 mg/kg/day in females).

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.

870.3100 90-Day Oral Toxicity – Mouse

Refer to carcinogenicity study.

870.3150 90-Day Oral Toxicity – Dog

Executive Summary: In a subchronic oral toxicity study (MRID 42248611, 44807215), fluazinam (98.5% a.i.) was administered to 4 beagle dogs/sex/dose daily via gelatin capsule at doses of 0, 1, 10 or 100 mg/kg/day for 90 days. Slides of brain tissue were later reexamined (Addendum to the original report, MRID 44807215) to assess vacuolation of white matter according to criteria for severity used in mouse carcinogenicity studies.

At 100 mg/kg/day, retinal effects of slight hyper-reflection and slight-to-moderate grey mottling of the tapetal fundus in all males and females during at least two of the three ophthalmologic examinations (7, 10 and/or 13 weeks), increased serum alkaline phosphatase levels (~2-fold, largely due to 1 female), increased SGPT in 1 female (~2 to 3-fold), increased absolute/relative liver weights (males 31%/34% and females 33%/36% above controls), hepatic coagulative necrosis (1 male, focal; 2 females, multifocal, all slight, vs. 0, controls) and slight to moderate bile duct hyperplasia with/without cholangiofibrosis (2 males and 2 females) were observed. A possible marginal increase in cerebral white matter vacuolation was observed in the reevaluation reported in the Addendum (1 male and 2 females, severity graded as trace vs. 0, controls). There were no treatment-related effects observed for clinical signs of toxicity, body weight/weight gain, food consumption, hematology or urinalysis parameters or gross pathology. No treatment-related findings were observed at 1 or 10 mg/kg/day. **The LOAEL is 100 mg/kg/day, based on retinal effects, possible increased serum alkaline phosphatase in females, increased relative liver weight and liver histopathology and possible marginal increase in vacuolation of the cerebral white matter. The NOAEL is 10 mg/kg/day.**

This study is classified **Acceptable/Guideline** and satisfies the guideline requirement for a subchronic oral toxicity study [OPPTS 870.3150(§82-1b) in the dog.

870.3200 21/28-Day Dermal Toxicity – Rat

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)].

870.3465 28- 90-Day Inhalation – Rat

No subchronic inhalation study is available. A 28-day study has been requested.

A.3.2 Prenatal Developmental Toxicity

870.3700a Prenatal Developmental Toxicity Study – Rat

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.

Maternal Toxicity. 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.**

Developmental Toxicity. 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.

Non-guideline Developmental Range-Finding Toxicity - Rat

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 sternebrae 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 (14^{th}) 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. Despite numerous deficiencies in the conduct of this study, an assessment of appropriate doses of the test article for the main developmental toxicity study was possible.

870.3700b Prenatal Developmental Toxicity Study - Rabbit

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.

Maternal Toxicity. 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.**

Developmental Toxicity. 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.

870.3700b Prenatal Developmental Toxicity Study - Rabbit

Executive Summary: In another developmental toxicity study (MRID 42248615, 42248614, 42248617), 20-24 presumed pregnant New Zealand White rabbits per group were administered Fluazinam (98.5% a.i, Lot No.: 8303-2) by gavage in 1% w/v aqueous methyl cellulose at doses of 0, 0.3, 1.0, and 3.0 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 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.

Four control group animals and one each in the 1.0 and 3.0 mg/kg/day groups died or were killed *in extremis* following observations of reduced food intake, reduced fecal output, weight loss, nasal discharge, tremors, inactivity, abdominal bloat, abnormal respiration, and/or soiled, stained, wet, or matted fur. Deaths were not considered to be treatment-related and may be related to disease or intubation error. One female each in the 0.3 and 3.0 mg/kg/day groups aborted and were subsequently sacrificed on GD 21 and 28, respectively, following weight loss. An additional control group female that exhibited weight loss and scouring was killed on GD 6 and categorized as "removed from study. No statistically or biologically significant differences in body weight or food consumption were noted. **Therefore, the maternal toxicity LOAEL is not identified, and the maternal toxicity NOAEL is greater than or equal to 3.0 mg/kg/day.**

There were no differences between the control group and the 0, 0.3, 1.0 or 3.0 mg/kg/day groups for number of corpora lutea, number of implantation sites, live fetuses/dam, pre- and post-implantation losses, or mean fetal weight. The percentage of small fetuses (weighing less than 32.0 g.) were 19.6% (8/16), 13.0% (4/15), and 20.8% (6/14 litters), respectively, for the 0.3, 1.0, and 3.0 mg/kg/day groups as compared to 4.1% (5/18 litters) for concurrent controls. However, these fetal incidence rates did fall within the historical control range of 0.0 to 30.4% (mean 13.36%). No other statistically or biologically significant differences between treated and control groups were noted.

The overall incidence rates for litters containing grossly abnormal fetuses in the 0, 0.3, 1.0 and 3.0 mg/kg/day groups were 2/18, 1/16, 0/15, and 1/14, respectively. No single malformation apparently occurred at a statistically significant incidence in the treated groups, and the incidence of total major malformations was also not significantly increased for any of the treated groups as compared to controls. However, this assessment may be flawed since data from two separate studies were combined and disease and intubation errors were clearly apparent in the data. It is unclear how disease differed in the two studies and how this may have impacted on study

interpretation. **Therefore, the developmental toxicity LOAEL was not determined, and the developmental toxicity NOAEL is greater than or equal to 3.0 mg/kg/day.**

This study is classified as **Unacceptable/Guideline** and does not satisfy the requirements for a developmental toxicity study 870.3700 (§83-3) in rabbits. At the high dose, significant maternal effects were not reported, and developmental toxicity did not occur. In addition, due to the small number of animals available for examination due to disease and intubation errors, an additional study was started at a later date and these data were later combined and presented in this report. The use of sick animals and the methods of combining different populations of animals into a larger study as noted in this study are not acceptable.

A.3.3 Reproductive Toxicity

870.3800 Reproduction and Fertility Effects - Rat

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, 42248618). One litter was produced in each generation. Mean premating doses were 1.5, 7.3, and 36.6 mg/kg/day, respectively for F_0 males and 1.7, 8.4, and 42.1 mg/kg/day, respectively for F_0 females. Mean premating doses were 1.9, 9.7, and 47.3 mg/kg/day respectively, for F_1 males and 2.2, 10.6, and 53.6 mg/kg/day, respectively, for F_1 females. F_1 adults were chosen from the F_1 pups and weaned onto the same diet as their parents. Animals were given test or control diet for 11 weeks before mating within the same dose group.

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

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

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

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.

A.3.4 Chronic Toxicity

870.4100a (870.4300) Chronic Toxicity – Rat

Executive Summary: In a chronic oral toxicity study (MRID 44839901, 44807213), technical grade fluazinam (95.3% a.i., Batch # 8412-20) was administered to 25 Crl:CD®(SD)BR rats/sex/dose in the diet at dose levels of 0, 25, 50, or 100 ppm for 104 weeks (0, 1.0, 1.9, or 3.9 mg/kg/day for males; 0, 1.2, 2.4, or 4.9 mg/kg/day for females). A four-week range-finding study in rats was also conducted using 0, 10, 50, 250, or 3000 ppm (MRID 44807213).

No clinical signs of toxicity were observed, and survival rates were unaffected by treatment. At study termination, survival rates for the 0, 25, 50, or 100 ppm groups were 32, 52, 28, and 36% for the males, respectively, and 72, 56, 72, and 52% for the females, respectively. No treatment-related effects on mean absolute body weights, body weight gain, food consumption, or water consumption were noted.

No treatment-related differences were observed in hematology analysis or urinalysis, and treatment-related clinical chemistry changes were limited to a transient increase in total serum cholesterol in high-dose females at Week 52 (154% of controls, $p<0.01$). Relative liver weights were increased in high-dose females at study termination (124% of controls; $p<0.01$), but were not accompanied by any histopathological correlates. The increased relative liver weights and transient increase in cholesterol were therefore not considered adverse. Treatment with fluazinam appeared to affect the testes. High-dose decedent males (those not surviving to study

termination) had an increased incidence of small and/or flaccid testes (11/16 or 69%, 8/16 or 50%, respectively;) as compared with decedent controls (4/17 or 24% for both) during macroscopic examination. Microscopic examination revealed a corresponding increased incidence of marked tubular atrophy in the testes of these 100 ppm decedent males (8/16; 50%) as compared with decedent controls (3/17; 18%); however, this increase was not statistically significant and did not show a dose-response when considering the average severity ranking for the decedent males (2.5, 3.4, 2.6, and 3.4 for the 0, 25, 50, and 100 ppm decedent males, respectively). When considering all 100 ppm males (decedent + terminal), macroscopic examination did not reveal an increased incidence of small and/or flaccid testes (16/25 vs. 16/25 controls), but the overall severity of testicular tubular atrophy was increased in the high-dose males (3.1) as compared with controls (2.6). Because testicular atrophy was also an effect noted in male rats following dietary treatment with 100 or 1000 ppm in another chronic toxicity/carcinogenicity study (MRID 42248620), it is considered an effect of treatment in this study.

At the doses tested, there was not a treatment-related increase in tumor incidences when compared to controls.

A LOAEL of 100 ppm (3.9 mg/kg/day) was identified for male rats based on increased testicular atrophy, with a corresponding NOAEL of 50 ppm (1.9 mg/kg/day). A LOAEL could not be identified for females. The NOAEL for females was therefore 100 ppm (4.9 mg/kg/day).

This chronic oral toxicity study in the rat is classified as **Acceptable/Guideline** and satisfies the Subdivision F guideline requirement for a chronic oral toxicity study [OPPTS 870. 4100 (§83-1a)] in rats.

Executive Summary: In a 4-week oral range-finding study (MRID 44807213), technical grade fluazinam (96.3% a.i., Batch # 8203) was administered to 10 CD (Sprague-Dawley) rats/sex/dose in the diet at dose levels of 0, 10, 50, 250, or 3000 ppm for 4 weeks (0, 1.0, 5.1, 26.4, or 302 mg/kg/day for males; 0, 1.1, 5.3, 25.9, or 309 mg/kg/day for females).

The following treatment-related effects were observed at 3000 ppm in both males and females: decreased body weight gain, decreased food consumption, increased serum phospholipid, increased total cholesterol, increased absolute liver weights, and increased relative liver/body weight ratios. In addition, increased incidences of histopathological findings were observed in the liver of males (increased periacinar hypertrophy) and in the liver of females (increased single cell necrosis). The following treatment-related effects were also observed at 250 ppm: decreased body weight gain (females), decreased food consumption (females), increased serum phospholipid (females), increased total cholesterol (males and females), increased relative liver/body weight ratios (females), and increased histopathology in the liver of males (increased periacinar hypertrophy). In an addendum to this study, there was no effect of treatment on the incidence or degree of white matter vacuolation in the brain of male or female rats of the high dose group (3000 ppm), compared with controls.

The LOAEL was 250 ppm (26.4 mg/kg/day for males; 25.9 mg/kg/day for females), based on decreased body weight gain (females), decreased food consumption (females), increased serum phospholipid (females), increased total cholesterol (males and females), increased

relative liver/body weight ratios (females), and increased histopathology in the liver of males (increased periacinar hypertrophy). The NOAEL was 50 ppm (5.1 mg/kg/day for males; 5.3 mg/kg/day for females).

This range-finding study is classified as **Acceptable/Nonguideline**. It does **not** satisfy the Subdivision F guideline requirement for a subchronic oral toxicity study [OPPTS 870.3100 (82-1)] in rats.

Executive Summary: In a combined chronic toxicity/carcinogenicity study (MRID 42248620, 44807223, 45150201), 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, 3.8, or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.9, 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.

No treatment-related effects were observed in rats receiving the 1 ppm or 10 ppm diets. No treatment-related effect on mortality was observed in rats receiving any dose of the test material. The only clinical signs observed were straw-discoloration of the fur in all rats receiving the 1000 ppm diet and an increased incidence of alopecia in females receiving the 1000 ppm diet.

Males receiving the 1000 ppm diet weighed 16% ($p<0.01$) less than controls from week 8 to study termination, gained 15% less weight overall, and consumed ≤8% less food than controls at each weekly interval. Females receiving the 1000 ppm diet weighed 24% ($p<0.01$) less than controls from week 2 to termination, gained 35% less weight overall, and consumed 18% less food than controls at each weekly interval. The food utilization factor or the food efficiency suggested that reduced body weight gain was due in part to toxicity of the test material. No treatment-related effects were observed on body weights, body weight gain, food consumption, or food utilization/efficiency in male or female rats receiving the 1-, 10- or 100-ppm diets. No treatment-related effects were observed on the eyes at any dose at any time during the study. Clinical pathology evaluations showed only mild anemia and elevated cholesterol in both sexes receiving the 1000 ppm dose. 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 acinal 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 ppm and 1000 ppm group males, and spermatocele granuloma also in 1000 ppm males. The incidence of sinus histiocytosis in the lymph nodes was increased in1000 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.

The lowest-observed-adverse-effect level (LOAEL) for fluazinam was 100 ppm (3.8 mg/kg/day for males and 4.9 mg/kg/day for females) based on liver toxicity in both sexes, testicular atrophy in males and pancreatic exocrine atrophy in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm (0.38 mg/kg/day for males and 0.47 mg/kg/day for females).

In this study, there were statistically significant positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/adenocarcinomas for the male rats. There was also a statistically significant increase by pair-wise comparison of the male high dose group (1000 ppm) with the controls for combined follicular cell adenomas/adenocarcinomas (23% vs. 8% in controls). In addition to an Exact Trend Test and a Fisher's Exact Test, a Peto's Prevalence Test was also conducted (which excluded animals that died or were sacrificed before observation of the first tumor at week 68). For follicular cell adenocarcinomas in males, results of the Peto's Prevalence Test showed a statistically significant positive trend and a borderline statistically significant ($p= 0.056$) increase by pair-wise comparison of the 1000 ppm male group with the controls (7% vs. 0% in controls), indicating the increased incidence of thyroid tumors had a malignant component to it. For combined follicular cell adenomas/adenocarcinomas, Peto's Prevalence Test also showed a statistically significant increase by pair-wise comparison of the high dose male group with the controls (26% vs. 9% in controls). The incidences of thyroid gland adenomas at 100 ppm and 1000 ppm (15% and 17%, respectively) and adenocarcinomas at 1000 ppm (6%) were slightly outside their respective ranges in the historical control data (range: adenomas, 0%-13%; adenocarcinomas, 0%-5%). Animals in the lower dose groups were not microscopically examined for thyroid lesions unless abnormalities were observed in that organ at gross necropsy. Therefore, percentage incidences of thyroid tumors in these lower dose groups may have been somewhat misleading (too high). The highest dose level tested in this study was considered to be adequate and not excessive because there were decreased body weight gains (up to 15% and 35% in males and females, respectively), decreased food consumption, decreased food efficiency, increased thyroid weights at 52 weeks, enlarged thyroids and a slightly increased incidence of thyroid gland follicular cell hyperplasia at 104 weeks in males. The survival of the animals was not decreased by treatment with the test material. There was no treatment-related increase in the thyroid tumor incidence in the female

rats in this study. 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 toxicologically significant increase in tumors was not observed in any other tissues in the treated male or female rats in this study.

This combined chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies guideline requirements for a chronic toxicity/carcinogenicity study [OPPTS 870.4300 (§83-5)] in rats.

870.4100b Chronic Toxicity – Dog

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 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.

A.3.5 Carcinogenicity

870.4200b Carcinogenicity (feeding) – Mouse

Executive Summary: In a carcinogenicity study (MRID 42208405, 44807220, 44807212), Fluazinam (95.3% a.i., lot no. 8412-20) was administered to groups of 52 male and 52 female CD®-1 mice in the diet at concentrations of 0, 0, 1, 10, 100, or 1000 ppm. There were 2 control groups. The test diets were given for 104 weeks. These concentrations resulted in mean daily compound intakes of 0.12, 1.1, 10.7, and 107 mg/kg/day for 1 ppm, 10 ppm, 100 ppm, and 1000 ppm, respectively, for males and 0.11, 1.2, 11.7, and 117 mg/kg/day, respectively, for females. Additional microscopic review of brain and spinal cord was presented in MRID 44807220. A four-week-range finding study (MRID 44807212) using 0, 10, 50, 250, or 3000 ppm in the diet was also conducted.

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

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

white matter vacuolation in the spinal cord was seen in either sex, and no treatment-related effects were seen at 1, 10, or 100 ppm.

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

In this study, there were statistically significant positive trends for hepatocellular adenomas, carcinomas and combined adenomas/carcinomas for the male mice. There were also statistically significant increases by pair-wise comparison of the male high dose group (1000 ppm) with the controls for hepatocellular adenomas (34% vs. 16% in controls), for hepatocellular carcinomas (34% vs. 19% in controls) and for combined hepatocellular adenomas/carcinomas (62% vs. 33% in controls). The incidence of hepatocellular adenomas (34%) at the highest dose level for males exceeded the highest incidence in the historical control data for 1981-1983 (4-27%) and for 1986-1988 (8-23%), and the incidence of hepatocellular carcinomas (34%) for the highest dose level for males exceeded the highest incidence in the historical control data for 1986-1988 (5-13%), but not for 1981-1983 (12-38%). There were no treatment-related tumors observed in the female mice in this study. The highest dose level tested was considered to be adequate but not excessive because liver and brain toxicity were observed in the male and female mice at 1000 ppm. Although there were no significant changes in survival or body weight gain, mean liver weight gains were increased in males and females and histopathological changes were observed in the livers and brain (vacuolation of the white matter) of males and females.

This carcinogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an carcinogenicity 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).

In a range-finding study (MRID 44807212), groups of 10 male and 10 female CD®1 mice were given concentrations of 0, 10, 50, 250, or 3000 ppm fluazinam (B-1216) in the diet for 4 weeks. There were no significant changes in body weights between treated and control animals; however, group mean body weight gain was slightly less in both sexes at 250 and 3000 ppm. The body weight change did not show a clear dose dependency especially in females. Platelet counts were marginally elevated in males at 3000 ppm and total blood cholesterol was slightly higher in both sexes at 3000 ppm compared to the controls. Phospholipid concentration was slightly increased in females and marginally increased in males at 3000 ppm. Blood glucose concentrations were increased in females at 250 and 3000 ppm compared to the control group. Absolute and relative (to body weight) liver weights were increased at 3000 ppm in both sexes and the absolute and relative kidney weights were increased in females at 3000 ppm. Hepatocyte periacinar hypertrophy incidences and severity were increased in both sexes at 3000 ppm compared to the control groups.

870.4200b Carcinogenicity (feeding) - Mouse

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

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

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

In this study, there were no statistically significant positive trends for hepatocellular adenomas, carcinomas or combined adenomas/carcinomas for the male mice. For the mid-dose male mice

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

This carcinogenicity study in the mouse is **Acceptable/Guideline** and does satisfy the guideline requirement for an carcinogenicity study [OPPTS 870.4200 (83-2b)] in mice.

Executive Summary: Treatment levels of 0, 3000, 5000, or 7000 ppm in the diet were given to mice in a 4-week study (MRID 44807211). Treatment-related changes were seen in the liver of mice at all treatment levels and kidney effects were seen in males at 5000 and 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the brain were seen at four weeks in males at 3000, 5000, and 7000 ppm and in females at 7000 ppm. Treatment-related increased incidences and severity of vacuolation of white matter of the spinal cord were also seen in males at 5000 and 7000 ppm.

The LOAEL for vacuolation of white matter in the brain of male mice in this four-week study was 3000 ppm (555 mg/kg/day; lowest dose level tested). A NOAEL was not demonstrated for this effect (i.e. NOAEL <555 mg/kg/day).

870.4300 Combined Chronic Carcinogenicity Study – Rat

EXECUTIVE SUMMARY: In a combined chronic toxicity/carcinogenicity study (MRID 42248620, 44807223, 451450201), 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, 3.8, or 40 mg/kg/day for males and 0, 0.05, 0.47, 4.9, 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.

No treatment-related effects were observed in rats receiving the 1 ppm or 10 ppm diets. No treatment-related effect on mortality was observed in rats receiving any dose of the test material. The only clinical signs observed were straw-discoloration of the fur in all rats receiving the 1000 ppm diet and an increased incidence of alopecia in females receiving the 1000 ppm diet.

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 ≤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 ≤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. No treatment-related effects were observed on body weights, body weight gain, food consumption, or food utilization/efficiency in male or female rats receiving the 1-, 10- or 100-ppm diets. No treatment-related effects were observed on the eyes at any dose at any time during the study. Clinical pathology evaluations showed only mild anemia and elevated cholesterol in both sexes receiving the 1000 ppm dose.

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 acinal 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 females 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 ppm and 1000 ppm group males, and spermatocele granuloma also in 1000 ppm

males. The incidence of sinus histiocytosis in the lymph nodes was increased in1000ppm 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.

The lowest-observed-adverse-effect level (LOAEL) for fluazinam was 100 ppm (3.8 mg/kg/day for males and 4.9 mg/kg/day for females) based on liver toxicity in both sexes, testicular atrophy in males and pancreatic exocrine atrophy in females. The corresponding no-observed-adverse-effect level (NOAEL) was 10 ppm (0.38 mg/kg/day for males and 0.47 mg/kg/day for females).

CARC Comments: In this study, there were statistically significant positive trends for thyroid gland follicular cell adenocarcinomas and combined follicular cell adenomas/adenocarcinomas for the male rats. There was also a statistically significant increase by pair-wise comparison of the male high dose group (1000 ppm) with the controls for combined follicular cell adenomas/adenocarcinomas (23% vs 8% in controls). In addition to an Exact Trend Test and a Fisher's Exact Test, a Peto's Prevalence Test was also conducted (which excluded animals that died or were sacrificed before observation of the first tumor at week 68). For follicular cell adenocarcinomas in males, results of the Peto's Prevalence Test showed a statistically significant positive trend and a borderline statistically significant (p= 0.056) increase by pair-wise comparison of the 1000 ppm male group with the controls (7% vs 0% in controls), indicating the increased incidence of thyroid tumors had a malignant component to it. For combined follicular cell adenomas/adenocarcinomas, Peto's Prevalence Test also showed a statistically significant increase by pair-wise comparison of the high dose male group with the controls (26% vs 9% in controls). The incidences of thyroid gland adenomas at 100 ppm and 1000 ppm (15% and 17%, respectively) and adenocarcinomas at 1000 ppm (6%) were slightly outside their respective ranges in the historical control data (range: adenomas, 0%-13%; adenocarcinomas, 0%-5%). Animals in the lower dose groups were not microscopically examined for thyroid lesions unless abnormalities were observed in that organ at gross necropsy. Therefore, percentage incidences of thyroid tumors in these lower dose groups may have been somewhat misleading (too high). The highest dose level tested in this study was considered to be adequate and not excessive because there were decreased body weight gains (up to 15% and 35% in males and females, respectively), decreased food consumption, decreased food efficiency, increased thyroid weights at 52 weeks, enlarged thyroids and a slightly increased incidence of thyroid gland follicular cell hyperplasia at 104 weeks in males. The survival of the animals was not decreased by treatment with the test material. There was no treatment-related increase in the thyroid tumor incidence in the female rats in this study. 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 toxicologically significant increase in tumors was not observed in any other tissues in the treated male or female rats in this study.

This combined chronic toxicity/carcinogenicity study in the rat is **Acceptable/Guideline** and satisfies guideline requirements for a chronic toxicity/carcinogenicity study [OPPTS 870.4300 (§83-5)] in rats.

A.3.6 Mutagenicity

Gene Mutation

Guideline 870.5100 Bacterial reverse mutation assay (Ames Test) with *S. typhimurium* and *E. coli* MRID 42270605 Acceptable	Negative with and without S9 up to cytotoxic concentrations. Compound was tested up 2µg/plate for *S. typhimurium* strains and up to 250 µg/plate for *E. coli* in the absence of S9, and up to 100 µg/plate for *S. typhimurium* strains and up to 500 µg/plate for *E. coli* in the presence of S9.
Guideline 870.5100 Bacterial reverse mutation assay (Ames Test) with *S. typhimurium* and *E. coli* MRID 42270604 Acceptable	Negative with and without S9 up to cytotoxic concentrations. Compound was tested up 1µg/plate for *S. typhimurium* strains and up to 250 µg/plate for *E. coli* in the absence of S9, and up to 100 µg/plate for *S. typhimurium* strains and up to 500 µg/plate for *E. coli* in the presence of S9.
Guideline 870.5300 Mammalian cells in culture forward gene mutation assay with mouse lymphoma L5178Y/TK +/- cells MRID 45156902 Acceptable	Negative with and without S9 up to 5 µg/mL. Compound was tested up to cytotoxic concentrations.
Guideline 870.5300 Mammalian cells in culture forward gene mutation assay with mouse lymphoma L5178Y/TK +/- cells MRID 45261801 Acceptable	Negative with S9 up to 9 µg/mL and without S9 up to 0.3 µg/mL. Compound was tested up to cytotoxic concentrations.

Cytogenetics

Guideline 870.5375 *In vitro* mammalian chromosome aberration (CHL cells) MRID 42270606 Acceptable	Negative with and without S9 up to cytotoxic concentrations. Compound was tested up 4 µg/mL in the absence of S9, and up to 9.5 µg/mL in the presence of S9. Cells harvested at 24 and 48 hours in nonactivated studies and at 24 hours in activated studies.

Guideline 870.5395 Mammalian erythrocyte micronucleus test (mouse) MRID 44807224 Acceptable	Negative. Test compound at 500, 1000, or 2000 mg/kg (oral gavage) with a 24 hour sacrifice or at 2000 mg/kg with 24, 48, and 72 hour sacrifices did not induce the formation of micronuclei in polychromatic erythrocytes from bone marrow. Clinical signs included piloerection, decreased motor activity, loose stools, and/or soiled fur.

Other Genotoxicity

Guideline 870.5550 Unscheduled DNA synthesis in primary rat hepatocytes MRID 45156901 Unacceptable	Negative; however, there were several serious study deficiencies: treatment time shorter than recommended, no data supporting the claim of cytotoxicity, and data variability for major endpoints.
Guideline 870.5500 Differential Killing/Growth Inhibition in *B. subtilis* MRID 42270607 Unacceptable	Negative without S9 up to 0.3 µg/disk and with S9 up to 30 µg/disk. Deficiency: only one replicate per plate dose was used.

A.3.7 Neurotoxicity

870.6200 Acute Neurotoxicity Screening Battery

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 ($\geq$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.

870.6200 Subchronic Neurotoxicity Screening Battery

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 fluazinam (MRID 44807217, 96.9%, Lot No. 6109) or 0, 1000, 2000, or 3000 ppm fluazinam (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 fluazinam. 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 (233 mg/kg/day) for male rats and 3000 ppm (280 mg/kg/day) for female rats. A neurotoxicity LOAEL was not identified.

These subchronic neurotoxicity studies are classified as **Acceptable/Guideline**. When considered together, these studies satisfy the guideline requirement for a subchronic neurotoxicity study [OPPTS 870.6200 (82-7ss)] in rats.

870.6300 Developmental Neurotoxicity Study

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (2005, MRID 46534401), Fluazinam (97.8% a.i.; lot # A629/1995, impurity #5 0.09%) was administered by gavage to 24

Crl:CD® (SD) IGS BR rats/sex/dose at 0, 2, 10 or 50 mg/kg/day from gestation day (GD) 6 through lactation day (LD) 20. The pups were administered the same doses by gavage from postnatal days (PND) 7 to 20 or 21. Maternal evaluation consisted of a Functional Operational Battery (FOB) was performed on GDs 12 and 18, and on post partum days (PPDs) 35, 45 and 60. Additional behavioral assessments included: motor activity on GD 15 and PPD 60; auditory startle habituation on GD 19 and PPD 58; and learning and memory on LD 16 and PPD 61. From each maternal group, 12 were sacrificed on both LD 21 and PPD 66; of these, 10/group were selected for neuropathology procedures. The additional assessment of the dams is related to trying to further characterizing neurotoxicity that is attributed to impurity #5. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (watermaze testing), and neuropathology at days 23/24 and on PND day 66. On PND 21, the whole brain was collected from 10 pups/sex/dose group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

Maternal parameters. No effects on absolute body weight were noted. Mean body weight *gain* for GDs 6-14 was significantly decreased (14%) at 10 and 50 mg/kg/day and during GDs 6-20 at 50 mg/kg/day (10%). Mean body weight gain during lactation was not affected. Food consumption during gestation was comparable to controls but during lactation was significantly decreased at 10 (10 to 14%) and 50 mg/kg/day (7 to 11%). Mean body weight and body weight gain post weaning (days 28-63) were not affected. The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be of sufficient magnitude to be included as a true toxic response. Reproductive parameters and behavioral assessments, including FOB, motor activity, auditory startle reflex habituation and learning and memory, were not affected by treatment. No treatment-related changes were observed at either the necropsy on LD 21 or PPD 66. The characteristic grey matter vacuolation attributed to fluazinam in previous studies was not seen at LD 21 or PPD 66. **The maternal LOAEL for Fluazinam was not established. The maternal NOAEL is > 50 mg/kg/day.** The weight *gain* and food consumption data that occurs in the absence of absolute weight differences for the dams are not considered to be sufficient of sufficient magnitude to be included as a true toxic response.

Offspring parameters. No treatment-related effects were observed on litter size at birth or survival to weaning. Birth weight was lower in females at 10 and 50 mg/kg/day (both 6%, $p < 0.01$) but not in males. Mean offspring body weight was significantly decreased in males and females at 10 (6-11%) and 50 mg/kg/day (6-16%) during lactation. Mean body weight gain was significantly decreased in male and female pups at 10 (4-24%) and 50 mg/kg/day (16-35%) during lactation. During the post-weaning period (PNDs 28-63), mean body weight was significantly decreased in males and females at 10 (3-7%) and 50 mg/kg/day (7-15%). However, post weaning body weight gain was essentially comparable to the control group. The mean age of completion of balano-preputial separation was significantly delayed at 10 and 50 mg/kg/day. Rearing counts in female pups were decreased on day 21 in the 10 (to a mean of 3.5 vs. 8.1 in the controls) and 50 (mean 3.7) mg/kg/day dose groups. Dark and/or distended abdomens observed in a total of 12 offspring from 4 litters at 50 mg/kg/day were considered treatment-related since similar signs were observed in the preliminary study. The peak amplitude in the auditory startle response was affected in males in the high dose group at day 23/24. Absolute brain weight for

high dose males was 6.1% decreased on PND 21 and slight changes in brain width were noted. No treatment-related effects were observed on other behavioral assessments, including FOB, motor activity or learning and memory. Grey matter vacuolation was not seen in the LD21 neuropathology assessment. A single isolated incident of grey matter vacuolation in the high dose group at day 66 was not considered related to treatment. **The offspring LOAEL is 10 mg/kg/day based on decreased body weight and body weight gain and delay in completion of balano-preputial separation. The offspring NOAEL is 2 mg/kg/day.**

This developmental neurotoxicity is classified as **Acceptable/Non-Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6): OECD 426 (draft) due to the pending review of the positive control data.

A.3.8 Metabolism

870.7485 Metabolism – Rat

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

There were no treatment-related deaths in the rats. Overall recovery of the administered radioactivity (reported in MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER) was acceptable (93.10-103.55%). Excretion via the urine was minor. AMPA mercapturate and DAPA, the major urinary metabolites, represented only 0.05-0.39% of the administered dose. Radioactivity in the feces represented most of the administered dose (88.78-100.03%) as determined by review of MRID Nos. 43521006, 43521007, and 43521008 and evaluated in a separate DER. Identified fecal metabolites, however, represented only 11.20-68.59% of the administered dose. For all dose groups, most of the fecal radioactivity appeared to reside with unextractable components in the post-extraction solids (PES). Further analysis of the PES components using base hydrolysis indicated that most of this radioactivity could be attributed to hydrolysis products of AMPA and DAPA. PES radioactivity was also greatest for the low-dose group which was consistent with the lower overall accounting of identified metabolites for this group. Approximately 20-25% of the aqueous phase of the fecal extraction was identified as a cysteine conjugate of DAPA and represented <1% of the administered dose. With the exception of the low-dose group, parent compound represented most of the identified radioactivity in the feces. AMPA and DAPA were identified in the feces from all dose groups but these metabolites never represented more than 5% of the administered dose (except for high-dose female rats where AMPA accounted for 10.22%).

DAPA glucuronide and AMPA mercapturate were the major biliary metabolites but represented <4% of the administered dose. Total biliary radioactivity, however represented 25-34% of the

administered dose (MRID Nos. 43521006, 43521007, and 43521008 evaluated in a separate DER). Analysis of chromatograms indicated that numerous other metabolites were present in the bile but were individually of insufficient quantity to allow for characterization.

Metabolite profiles from administration of different label positions (pyridyl and phenyl) indicated that there was no metabolic cleavage of the ring structures. Minor quantitative differences in metabolite recovery were observed between genders but not of sufficient magnitude to suggest biologically relevant differences in the metabolism of IKF-1216.

This metabolism study is **Acceptable/Guideline.** When considered together with the previously submitted general metabolism studies on IKF-1216 (MRIDs 43521004 through 43521008 and MRID 43553001), the requirement for a general metabolism study in rats [OPPTS 870.7485 (§85-1)] is satisfied.

870.7600 Dermal Absorption
There is no dermal absorption study with fluazinam and no study has been requested.

A.3.9 Special/Other Studies

Nonguideline 90-Day Oral Toxicity – Rat (Special Liver Toxicity Study)

Executive Summary: In a 90-day oral toxicity study (special liver toxicity study) (MRID 42248609), groups of 10 male and 10 female CD rats were given Fluazinam (a.i. 98.5%, Lot/Batch # 8303-2) administered at 0 or 500 ppm (equivalent to average mg/kg/day levels of 0 and 37.63 in males and 0 and 44.71 in females) in the diet. An additional group of 10 male and 10 female rats were given Fluazinam at 0 or 500 ppm in the diet for 90 days, then given no Fluazinam for 4 weeks in a reversibility study.

No deaths or adverse clinical signs occurred during this study. There were no treatment-related changes in body weight, body weight gain, food consumption or food efficiency. Microsomal aminopyrine-N-demethylase activity and absolute liver weights were not affected by Fluazinam. Observed treatment-related changes in the liver were limited to increased relative liver/body weight during the feeding phase in both males (+12%, $p \leq 0.01$) and females (+15%, $p \leq 0.01$), and periacinar hepatocytic hypertrophy in 10/10 males (0/10 controls). These effects resolved after the 4-week reversibility phase.

This special liver toxicity study in rats is classified as **Acceptable /Nonguideline.** The study did not fully meet its objective of assessing the hepatotoxic effects of the test material or determine their reversibility because only one dose (500 ppm) of Fluazinam was utilized, and the modest liver changes observed were of questionable toxicological significance.

Nonguideline 11-Week Oral Toxicity – Dog (Special Retinal Toxicity Study)

Executive Summary: In an 11-week oral toxicity study (special retinal toxicity study) (MRID 44807216), Fluazinam (2 batches from Lot no. 8303-2: 98.0% and 98.1% a.i.) was administered in gelatin capsules for 11 weeks to 6 male beagle dogs/group at 0 or 200 mg/kg/day (dose reduced to 150 mg/kg/day in two animals at week 3, and in the remaining four animals at week 5, due to excessive toxicity). Three animals/dose group were terminated on study day 77

(Group II; corresponding controls Group I), and the remaining 3 animals/group remained on study without treatment for 5 weeks before termination on study day 112 (Group IIW; corresponding controls Group IW). This study was designed to evaluate retinal changes seen at 100-150 mg/kg/day in previous 28- and 90-day studies, follow the time course of any changes, assess retinal function and assess reversibility. In addition to ophthalmologic evaluation, fundic photography and electroretinography were performed pretest, weekly during treatment and at weeks 2 and 4 of compound withdrawal. Eyes were examined microscopically and by electron microscopy.

At 150 mg/kg/day (maximum tolerance level), dogs experienced frequent loose/liquid feces, vomiting, inappetence, excess salivation, vasodilation (as noted by pink or reddened ears and/or abdomens); these findings were observed sporadically or not at all in controls. Unusual behavioral (2 males) and motor changes (1 male)were observed on the first day of dosing only. Decreased mean body weight/weight gains during treatment (at termination of treatment, -15%/-89% less than corresponding control group, Group II; -6%/weight loss of -0.61 kg, Group IIW), were also observed. Food consumption was also decreased during treatment (mean estimated at >10%, Group II and >6%, Group IIW; see Results for details) but not withdrawal (measurements were not possible for several weeks due to mixing with unspecified amounts of supplemental diet). Mean ALP, ALT, AST and blood urea levels were elevated due to increases above the provided reference range in 1-2 animals/dose group. Instead of grey mottling of the retinal tapetal fundus noted in previous studies, increased brown granularity of the tapetal fundus was observed in the tapetal fundus of one main study animal and in one withdrawal group treated animal, although this effect was too subtle to be demonstrated photographically. Electroretinography (ERG) demonstrated that dosing was associated with decreased a- and b-wave amplitudes of ~50%, but waveform was not altered. Other measurements of scotopic (dark or dim light) vision were not significantly altered and there was no evidence of neural damage. The ERG amplitudes in 2/3 treated animals recovered to some degree during withdrawal.

This eleven-week oral toxicity in the dog is **Unacceptable/Nonguideline (not upgradeable)**. This study did not repeat the ophthalmologic findings observed in the previous 28- and 90-day studies (grey mottling of the retina), although this did not necessarily render this special study results invalid and brown granularity in the retina was observed in 2 treated dogs. However, several factors compromised the integrity of the study: unexplained incongruity in the ERG data between control groups and between the control and treated animals and other technical problems in the ERG evaluation, double-penning of the animals and the possibility of illness due to an infectious agent, unspecified technical problems, errors and uncertainties in food consumption data, a striking parallelism of mean body weights for control and treated animals and unexplained decreased gain in the withdrawal control animals. Despite these problems, the study does appear to support the conclusion that the retina is a target tissue for fluazinam and that functional as well as morphological alterations may result from exposure.

Nonguideline 7-Day Inhalation Toxicity (Range-Finding)- Rat
(Test Material: Frowncide® WP (51.9% fluazinam, a.i.)

Executive Summary: In a 7-day inhalation toxicity (range-finding) study (MRID 42248621), groups of five male and five female young adult CD rats were exposed nose-only to Frowncide® 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

Frowncide® 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/Nonguideline.** 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.

Nonguideline: A Review of 8 Special Mechanistic Studies Conducted to Assess the CNS White Matter Vacuolation Produced by Impurity-5 Present In Fluazinam Technical

Background: 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) 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, and 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 mechanistic studies, however, demonstrated the lesion could also be induced in rats at higher dose levels than used in the guideline studies. These 8 additional special mechanistic studies were designed to determine, if possible, the etiology of the vacuolation of the white matter of the CNS observed in the guideline studies on fluazinam and to further evaluate several additional characteristics of the lesion.

Summary: Eight special studies (MRIDs 44807225, 44807226, 44807227, 44807228, 44807229, 44807230, 44807231, and 44807232) conducted on the etiology of an impurity in Fluazinam Technical which produced vacuolation of the white matter in the CNS of mice, rats, and dogs have revealed several important toxicological features. In the mouse, rat, and dog, vacuolation of the white matter in the brains (and optic nerves of mice) was observed only when high doses of fluazinam technical were administered. Fluazinam technical itself was not responsible for the induction of this aberration. An analysis of the effects of nine impurities present in Fluazinam Technical revealed that one impurity, Impurity-5, is responsible for the appearance of white matter vacuolation. 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. No significant differences between species/sex susceptibility were observed. White matter vacuolation in the CNS was reversible, and no progression of this abnormality was observed with time. An age-related increased sensitivity was identified in mice and rats at 10 weeks compared to 3 weeks of age. 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. Macroscopic changes in the liver of animals treated with fluazinam technical and the analytical standard of fluazinam revealed that liver effects are due to fluazinam itself and not to the presence of Impurity-5.

Review: A brief synopsis of the results in the special mechanistic studies is presented below.

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

   Fluazinam, *per se*, was not responsible for the induction of this lesion. An analysis of the effects of nine impurities present in fluazinam 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 fluazinam technical used for toxicity testing at concentrations ranging from <0.005% to 0.2% w/w. 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.

   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 fluazinam (containing <0.0005% Impurity-5) caused no vacuolation of the white matter of the brain whereas similar doses of fluazinam technical did cause vacuolation of the white matter of the brain.

2. Determination of dose-response relationships for fluazinam 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 fluazinam technical were administered. In mice, single oral (gavage) doses of 3000 mg/kg of fluazinam 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 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 that 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 0.1% w/w [see memorandum from Indira Gairola, Technical Review Branch, RD (7505C) to Cynthia Giles-

Parker, Fungicide Branch, RD (7505C), dated May 18, 2001, DP Barcode D272455], this is equivalent to:

NOAEL (for CNS effects) = 20.0 mg/kg/day of technical grade fluazinam

Calculation: $\frac{0.02 \text{ mg/kg/day}}{0.1\%} = \frac{x \text{ mg/kg/day}}{100\%}$ $x = 20.0$

This NOAEL (for CNS effects) of 20.0 mg/kg/day for technical grade fluazinam is to be compared to:

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

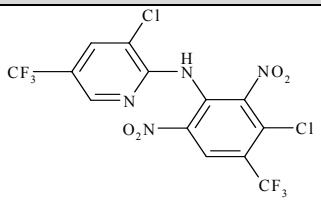
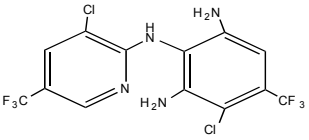
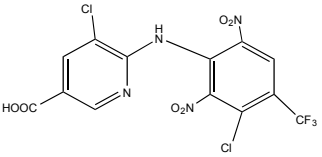
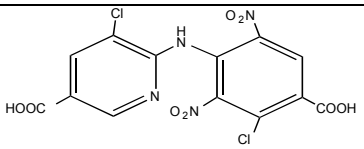
The chronic RfD of 0.011 mg/kg/day for "all populations", including infants and children, is therefore protective of the CNS effects caused by Impurity-5 present in technical grade fluazinam at levels up to 0.1% w/w.

A.4 Additional Toxicology Study

Fulcher, S. (2005) Technical Fluazinam: Developmental Neurotoxicity Study in the Rat by Oral (Gavage) Administration. Project Number: ISK/272/042019. Unpublished study prepared by Huntingdon Life Sciences, Ltd. 1471 p.

Appendix B: Metabolism Assessment

B.1. Metabolism Guidance and Considerations

Chemical Name (other names in parenthesis)	Matrix	Structure
Parent Fluazinam	Primary crop, Ruminant, Rat, and Drinking water	
*AMGT	Primary crop (AMGT data only available for blueberries)	Not available
AMPA	Ruminant, Rat (+ AMPA hydrolysis products)	Not available
DAPA	Ruminant, Rat (+ DAPA hydrolysis,conjugation products), Drinking water	
CAPA	Drinking water	
DCPA	Drinking water	

Appendix C: Tolerance Reassessment Summary and Table

TABLE C.1 Tolerance Summary for Fluazinam.			
Commodity	**Proposed Tolerance (ppm)**	**Recommended Tolerance (ppm)**	**Comments [*Correct Commodity Definition*]**
Ginseng	3.00	4.5	
Bean, dry	0.01	0.02	[*Pea and bean, dried shelled, except soybean, subgroup 6-C (except peas)*]
Succulent-shelled legume vegetables subgroup 6B, except pea	0.02	0.04	[*Pea and bean, succulent shelled, subgroup 6-B (except peas)*]
Edible-podded legume vegetables subgroup 6A, except peas	0.15	0.10	[*Vegetable, legume, edible-podded, subgroup 6-A (except peas)*]
Leafy *Brassica* greens subgroup	0.02	0.01	Crop group tolerance is appropriate. [*Vegetable, Brassica leafy, group 5*]
Head and stem *Brassica* subgroup	0.01		
Turnip, leaves	0.02	0.01	[*Turnip, tops*]
Bushberry subgroup 13B	4.5	7.0	[*Bushberry subgroup 13-B*]
Aronia berry	4.5	7.0	
Blueberry, lowbush	4.5	Not needed	Low bush blueberry is a member of the bushberries subgroup 13-B
Buffalo currant	4.5	7.0	
Chilean guava	4.5	7.0	
European barberry	4.5	7.0	
Highbush cranberry	4.5	7.0	
Honeysuckle	4.5	7.0	
Jostaberry	4.5	7.0	
Juneberry	4.5	7.0	
Lingonberry	4.5	7.0	
Native currant	4.5	7.0	
Salal	4.5	7.0	
Sea Buckthorn	4.5	7.0	