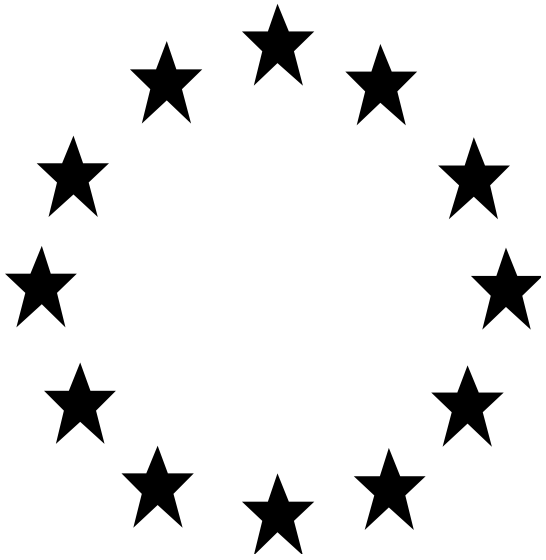

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State Austria for the existing active substance

FLUAZINAM

of the third stage (part A) of the review programme referred to in Article 8(2) of Council Directive 91/414/EEC

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Annex B

Fluazinam

B.6 Toxicology and metabolism

B.6 Toxicology and metabolism

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

B.6.1.1 Oral ADME studies in the rat

Several studies dealing with adsorption, distribution, metabolism and excretion of fluazinam in rats have been submitted. Although no specific test guidelines are cited in them, the studies fullfill the requirements of the OECD guideline 417 and were performed in compliance with GLP principles; they are scientific valid and acceptable.

Pilot study to evaluate the excretion of radiolabel following a single oral dose of ^{14}C-IKF-1216 to rats

Reference: *Liu Y.; 1993;* Report No. 5204-92-0034-AM-001

Material and Methods:

^{14}C-fluazinam (lot number 0201, purity 99.9 %), suspended in 0.75% methylcellulose (w/v), was administered by oral gavage to three rats (strain: Sprague-Dawley Crl:CD BR VAF/Plus) per sex per dose level per label position. Dose levels of 0.5 mg/kg and 50 mg/kg for the ^{14}C-Phenyl (^{14}C(B)) and ^{14}C-Pyridine (^{14}C(PY)) labeled test material were used. A total of two animals of each sex received the vehicle, 0.75 % methylcellulose, to serve as a control to provide sample for determination of efficiencies and background radioactivity. Urine, feces, expired air and volatile organic materials were collected over a 48-hour period and analyzed for radioactivity. Cage washes were collected at termination and also tested for radioactivity. The gastrointestinal tracts plus contents were excised and analyzed for radioactivity from animals terminated at 48 hours. Extracts of feces were analyzed by HPLC.

Findings:

Results of analysis indicated that recovery appeared to be independent of sex, dose level or label position with 86 % – 103 % of the administered dose being recovered. Feces, the major route of elimination, contained 86 % - 94 % of the administered ^{14}C(B) label and 57 % - 98 % of the administered ^{14}C(PY) label. An average of > 95 % of the recovered radiolabel was found in excreta by 48 hours after dose administration. Urine was a minor excretory route and contained 1 % – 3 % of the administered dose. Less than 0.1 % of the administered dose was found in expired air (table 6.1.1-1).

HPLC analysis data indicated that the profiles of radioactivity extracted from the feces are the same for the two label positions, so the use of a single label may be sufficient to define the animal metabolism of fluazinam.

Preliminary mass spectroscopic data suggested that the parent compound and two metabolites (mono and diamino analogs of parent compound) are present in ether extracts of feces regardless of label position.

Conclusion:

The excretion/elimination parameters of ^{14}C-fluazinam were the same regardless of sex,

dose level or label position.

The major route of elimination was the feces. Urine was a minor excretory route and the radiolabel was practically not expired in air.

HPLC profiles of radioactivity extracted from feces indicate that only one label position may be needed to define the animal metabolism of fluazinam.

Table 6.1.1-1: Summary of administered radiolabel recovered (% of administered dose)

Dose mg/kg	Sex	Exp. Air	Urine	Feces	GI tract	Cage Wash	Total recovered
Phenyl label							
50	Male	0.04	2.17	86.48	1.38	2.69	92.76
50	Female	0.04	1.76	90.69	1.05	4.86	98.40
0.5	Male	0.03	1.16	92.75	1.32	1.51	96.77
0.5	Female	0.02	2.73	93.88	1.93	0.37	98.94
Pyridine label							
50	Male	0.06	2.94	56.98	19.77	3.69	83.44
50	Female	0.08	2.67	79.15	1.81	5.03	88.73
0.5	Male	0.10	1.63	94.37	4.64	0.88	101.62
0.5	Female	0.13	2.93	98.43	1.28	1.09	103.85

Study to measure the pharmacokinetics of phenyl-^{14}C-IKF-1216 in the blood of rats

Reference: *Andre J. C.; 1994;* Report No. 5319-92-0262-AM-001

Material and Methods:

^{14}C-fluazinam (lot number 0201, purity 99.9 %), suspended in 0.75% methylcellulose (w/v), was administered by oral gavage to five rats (strain: Sprague-Dawley Crl:CD BR VAF/Plus) per sex per dose level. Dose levels of 0.5 mg/kg and 50 mg/kg of the ^{14}C-Phenyl (^{14}C(B)) labeled test material were used. Two animals of each sex received the vehicle, 0.75 % methylcellulose, to serve as a control for determination of background radioactivity. Blood was collected by orbital sinus puncture at 0.25, 0.50, 0.75, 1, 2, 4, 6, 8, 10, 12, 14, 18, 24, 48 and 72 hours. All samples were analyzed for radiolabel content. Animals were terminated at 72 hours.

Findings:

The median peak time for blood concentration of radiolabel activity for both sexes was six hours. At the time of peak concentration, the radioactivity in the blood represented about 0.4 % of the Administered Dose (AD) for the 50 mg/kg dose level groups and 0.5 % to 0.6 % AD for the 0.5 mg/kg groups. There were no significant differences in peak concentration between sexes in either dose group. By 72 hours, about 0.1% AD was found in the blood for both sexes at both dose levels. No sex-related differences in half-life were found in either dose group. The elimination rate at the high dose remained relatively constant throughout

the study. At the high dose, the half-life for males was 32 hours and 27 hours for females. Since the elimination rates for the low dose animals were biphasic, half-lives were determined from the peak time to 18 hours (α phase) and from 18 or 24 hours to 72 hours (β phase). The half-lives for the α phase were 5.4 hours for males and 4.5 hours for females and for the β phase were 42 hours for males and 39 hours for females. The half-lives for low- and high-dose animals were significantly different at the 0.05 level, but were not different between sexes at each dose level. There were no significant differences between sexes in either dose group for the area under the blood concentration versus time curve (AUC). The AUC ratios of high- to low-dose animals were directly proportional to the dose ratio.

Conclusion:

The median peak time for blood concentration of radiolabel activity for both sexes was six hours, representing approximately 0.4 % - 0.6% of the administered dose. 72 hours post dosing, approximately 0.1 % of the administered dose was found in the blood of all animals.

<u>Study to evaluate the distribution and excretion of (Phenyl-^{14}C)-IKF-1216 ^{14}C(B)-IKF-1216 in rats</u>

<u>Reference:</u> *Andre J. C.; 1994;* Report No. 5304-92-0185-AM-001

Material and method:

^{14}C-fluazinam (lot number 0201, purity 99.9 %), suspended in 0.75% methylcellulose (w/v), was administered by oral gavage to ten rats (strain: Sprague-Dawley Crl:CD BR VAF/Plus) per sex per dose level. Dose levels of 0.5 mg/kg bw and 50 mg/kg bw of the ^{14}C-Phenyl (^{14}C(B)) labeled test material were used. Two animals of each sex received the vehicle, 0.75 % methylcellulose, to serve as a control for determination of background radioactivity. Urine and feces were collected throughout the course of the study and analyzed for radioactivity. Subsequent collections were at 12 – 24, 24 – 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144 and 144 – 168 hours. Animals were sacrificed by exsanguination after 168 hours. At termination, cages were rinsed with water and methanol, the washes combined and analyzed. Blood was collected from the dorsal aorta. Heart, lung, spleen, liver, kidney, gonads, gastrointestinal tract, hind leg muscle, mesenteric fat, brain, bones and residual carcass were taken for analysis.

Findings:

<u>Rate and extend of excretion:</u> Following single oral administration of ^{14}C-fluazinam, the majority of radioactivity was eliminated in feces within the first 48 hours following dosing (> 95 %).

At termination, the recovery of radioactivity in feces of male rats was 93.9 % ± 3.61 % of the administered dose, the recovery in feces of female rats was 88.78 % ± 2.26 % of the administered dose at the low dose level. At the high dose level, male feces contained 94.23% ± 9.52 %, female feces contained 91.6 % ± 5.11 % of the administered dose.

Urine was a minor excretory route. The recovery of radioactivity in urine of male rats of the low dose group was 2.16 % ± 0.72 % of the administered dose over the 7 day collection

period. In females, 4.32 % ± 0.56 % of the radioactivity was eliminated via urine. At the high dose groups, recovery of radioactivity in urine of male rats was 3.97 % ± 4.71 %, of female rats 3.26 % ± 2.02 % of the administered dose.

The mean recovery of radioactivity in the cage wash ranged from 0.04 to 0.74 % of the administered dose over the 7 day collection period (table 6.1.1-2).

Table 6.1.1-2: Group mean recovery of radioactivity in urine, feces, cage wash and tissues (mean % of radioactive dose, 168 hour collection period) after single oral application of (14C)-fluazinam to rats

	low male	low female	high male	high female
Feces	93.90	88.78	94.23	91.60
Urine	2.16	4.32	3.97	3.26
Cage wash	0.04	0.09	0.26	0.74
Tissues	0.55	0.59	0.50	0.47
Total recovery	96.66	93.78	98.96	96.07

Distribution: Radioactivity in the sampled tissues was highest in carcass, liver and muscle expressed as a percent of the administered dose (up to 0.34 %, 0.19 %, 0.13 %) respectively. Tissue data expressed as nanogram equivalents per gram of tissue gave the highest value in liver (mean value of 14.12 ng equiv/g in males and 12.50 ng equiv/g in females of the low dose groups respectively, 1510 ng equiv/g in males and 1070 ng equiv/g in females of the high dose groups). (table 6.1.1-3).

Table 6.1.1-3: Radioactivity in the tissues, mean values, expressed as ng equivalents/g (% of administered dose) 168 hours after single oral application of (14C)-fluazinam to rats

Tissue	Concentration of radioactivity, ng equivalents/g (% of AD)			
	Male		Female	
	low	high	low	High
Whole blood	1.45 (0.03)	66.10 (0.01)	2.88 (0.04)	113.09 (0.01)
Gastrointestinal tract	2.57 (0.08)	241.67 (0.07)	2.76 (0.06)	234.98 (0.05)
Carcass	1.21 (0.23)	95.77 (0.20)	2.07 (0.34)	154.01 (0.26)
Bone	0.78 (0.01)	82.01 (0.01)	1.06 (0.01)	89.32 (0.01)
Brain	0.66 (0.00)	64.92 (0.00)	1.11 (0.00)	125.36 (0.00)
Fat	2.92 (0.05)	229.71 (0.05)	4.43 (0.07)	435.29 (0.07)
Heart	1.37 (0.00)	119.23 (0.00)	3.57 (0.00)	284.29 (0.00)
Kidney	7.99 (0.02)	820.94 (0.02)	13.11 (0.02)	863.79 (0.01)
Liver	14.12 (0.18)	1510.32 (0.19)	12.50 (0.12)	1069.74 (0.11)
Lung	1.37 (0.00)	143.83 (0.00)	2.58 (0.00)	230.56 (0.00)
Muscle	1.00 (0.13)	56.97 (0.07)	1.28 (0.13)	134.48 (0.13)
Spleen	1.50 (0.00)	112.79 (0.00)	2.90 (0.00)	163.89 (0.00)

Gonads	1.76 (0.00)	73.2 (0.00)	4.58 (0.00)	309.61 (0.00)

Conclusion:

Recovery of administered radioactivity was approximately 96 % of the dose and was independent of dose level and sex. The majority of the administered dose was eliminated in the feces which contained an average of greater than 88 % of the doses by both sexes at both dose levels. Urine was a minor excretory route and accounted for an average of 2 to 4 % of the dose. Less than 1 % of the dose was found in any animal 168 hours after dose administration. The highest concentration of radiolabel at both dose levels was found in the liver.

Study to evaluate the distribution and excretion of (Phenyl-^{14}C)-IKF-1216 in rats following repeated dosing

Reference: *Andre J. C.; 1994;* Report No. 5317-93-0021-AM-001

Material and method:

A dose of 0.5 mg/kg bw nonradiolabeled fluazinam (lot number 0301, purity 99.9 %), suspended in 0.75% methylcellulose (w/v), was administered by oral gavage to two sets of five rats (strain: Sprague-Dawley Crl:CD BR VAF/Plus) per sex for 14 consecutive days (ten rats total/set). On day 15, a suspension of ^{14}C-fluazinam (labelled in the phenyl position) in 0.75% methylcellulose (w/v) was administered by oral gavage to the animals. Two animals of each sex received the vehicle, 0.75 % methylcellulose, to serve as a control for determination of background radioactivity. Animals were sacrificed by exsanguination either 24 or 168 hours after ^{14}C-fluazinam administration. Urine and feces were collected throughout the course of the study and analyzed for radioactivity. Collection containers were changed at 6, 12 and 24 hours following dose administration. Subsequent collections for the 168 hour set were 24 – 48, 48 – 72, 72 – 96, 96 – 120, 120 – 144 and 144 – 168 hours. At termination, cages were rinsed with water and methanol, the washes combined and analyzed. Blood was collected from the dorsal aorta. Heart, lung, spleen, liver, kidney, gonads, gastrointestinal tract, hind leg muscle, mesenteric fat, brain, bones and residual carcass were taken for analysis.

Findings:

Rate and extend of excretion: By termination at 24 hours after dosing, the recovery of radioactivity in feces of male rats was 91.73 % ± 7.01 %, the recovery in feces of female rats 83.83 % ± 5.80 % of the administered dose. Animals, which were maintained for 7 days after dosing, eliminated approximately 85 % during the initial 24 hours. By 7 days, male feces contained 93.49% ± 4.49 %, female feces 100.03 % ± 5.33 % of the administered dose. Urine was a minor excretory route. The recovery of radioactivity in urine of male rats after 24 hours was 1.16 % ± 0.10 %, in urine of female rats 2.84 % ± 0.64 % of the administered dose. At 7 days, recovery of radioactivity in urine of male rats was 1.36 % ± 0.20 %, of female rats 3.52 % ± 0.52 % of the administered dose.

Animals sacrificed 24 hours after dose administration retained from approximately 2 to 12 % of the administered dose in their gastrointestinal tracts (including contents). In male rats, the GI tracts plus contents represented 3.95 % ± 1.26 %, in female rats 7.55 % ± 4.15 %. 7 days after dosing, only 0.06 % ± 0.01 % remained in the GI tracts plus contents of either sex (table 6.1.1-4).

Table 6.1.1-4: Group mean recovery of radioactivity in urine, feces, cage wash, gastrointestinal tract and tissues (mean % of radioactive dose), 24 and 168 hours after the last dose administration of (14C)-fluazinam to rats

	24 hours male	24 hours female	168 hours male	168 hours female
Feces	91.73	83.83	93.49	100.03
Urine	1.16	2.84	1.36	3.52
Cage wash	0.48	0.21	0.06	0.08
Tissues	8.61	11.81	0.55	0.55
GI tract	3.95	7.55	0.06	0.07
Total recovery	101.99	98.69	95.45	104.19

Distribution: At the 24 hour termination, liver and fat contained the most radiolabel expressed as nanogram equivalents per gram of tissue. In the livers of male rats an average of 97 ng equiv/g and in the livers of females an average of 107 ng equiv/g were detected. The average concentration in fat was 126 ng equiv/g in males and 211 ng equiv/g in females. In kidneys, a concentration of 52 ng equiv/g in males and 61 ng equiv/g in females was measured. In all other tissues, with the exception of ovaries of females (43 ng equiv/g), no concentration exceeded 20 ng equiv/g.

7 days after dosing, liver and fat contained the most radiolabel also. The average concentration found in liver was 14 ng equiv/g in males and 12 ng equiv/g in females. An average of 10 ng equiv/g was measured in the fat of males, an average of 5.5 ng equiv/g in the fat of females. 6.5 ng equiv/g were found in kidneys of males and 8.8 ng equiv/g in kidneys of females. In all other tissues, no concentration exceeded 2.7 ng equiv/g.

The average concentration of radiolabel in the GI tract decreased from 180.65 ng equiv/g at 24 hours to 2.73 ng equiv/g at 168 hours in males and from 294.95 ng equiv/g at 24 hours to 2.55 ng equiv/g at 168 hours in females (table 6.1.1-5).

Table 6.1.1-5: Radioactivity in the tissues, mean values, expressed as ng equivalents/g (% of administered dose) 24 and 168 hours after the last dose administration of (14C)-fluazinam to rats

Tissue	Concentration of radioactivity, ng equivalents/g (% of AD)			
	Male		Female	
	24 hours	168 hours	24 hours	168 hours
Whole blood	12.07 (0.18)	1.02 (0.02)	15.44 (0.22)	2.01 (0.03)
Gastrointestinal tract	180.65 (3.95)	2.73 (0.06)	294.95 (7.55)	2.55 (0.07)
Carcass	19.23 (3.29)	1.51 (0.30)	16.95 (2.80)	1.55 (0.29)

Tissue	Concentration of radioactivity, ng equivalents/g (% of AD)			
	Male		Female	
	24 hours	168 hours	24 hours	168 hours
Bone	4.92 (0.07)	0.69 (0.01)	5.73 (0.08)	0.89 (0.01)
Brain	2.67 (0.00)	0.06 (0.00)	3.48 (0.01)	0.65 (0.00)
Fat	126.44 (2.03)	10.16 (0.19)	210.66 (3.36)	5.50 (0.10)
Heart	10.77 (0.01)	0.79 (0.00)	16.08 (0.01)	2.00 (0.00)
Kidney	52.19 (0.08)	6.45 (0.01)	61.26 (0.10)	8.82 (0.02)
Liver	97.24 (1.08)	14.04 (0.15)	107.25 (1.10)	12.24 (0.13)
Lung	14.51 (0.01)	1.28 (0.00)	17.10 (0.02)	2.01 (0.00)
Muscle	5.73 (0.59)	0.21 (0.03)	8.36 (0.85)	0.69 (0.08)
Spleen	9.91 (0.00)	1.09 (0.00)	9.07 (0.00)	1.56 (0.00)
Gonads	7.13 (0.01)	0.17 (0.00)	43.22 (0.00)	1.34 (0.00)

Conclusion:

Recovery of administered radioactivity was approximately 100 % of the dose and was independent of termination time and sex.

The majority of radioactivity was eliminated in feces by both sexes, which contained an average of approximately 84 % or more of administered dose at 24 hours and > 93 % at 7 days. Urine was a minor excretory route and accounted for an average of 1 to 3 % of the dose at 24 hours and 1 to 4 % of the dose at 168 hours.

Only carcasses, fat and livers of animals sacrificed 24 hours after dose administration contained 1 % or more of the dose. Concentrations in fat were highest followed by concentrations found in livers, concentrations in kidneys were about half those in livers. At 168 hours, concentrations of radioactivity in livers were 0.13 % - 0.15 %, concentrations in fat 0.10 % - 0.19 %. All other organs contained less than 0.1 %. These low levels of radioactivity found in tissues appear to be a reflection of the high elimination rates observed and indicate a low potential for bioaccumulation.

<u>Study of the biliary excretion of radiolabel following oral administration (Phenyl-^{14}C)-IKF-1216 to male Sprague-Dawley rats</u>

<u>Reference:</u> *Marciniszyn Ph. D. et al; 1995;* Report No. 5318-92-0321-AM-001

Material and method:

^{14}C-fluazinam (lot number 910801, purity 99.9 %), suspended in 0.75% methylcellulose (w/v), was administered by oral gavage to 6 or 7 male rats respectively (strain: Sprague-Dawley Crl:CD BR VAF/Plus) following cannulation of their bile ducts. Dose levels of 0.5 mg/kg (7 animals) and 50 mg/kg (6 animals) of the ^{14}C-Phenyl (^{14}C(B)) labeled test material were used. Two animals received the vehicle, 0.75 % methylcellulose, to serve as a control. Bile was collected continuously at 1 hour intervals for 48 hours. Urine and feces were

collected at 6, 12, 24 and 48 hours, cage washes were collected at termination. Animals were sacrificed at 48 hours, the gastrointestinal tract (GI) plus contents were excised for analysis separately from carcass. All samples were analyzed for radiolabel content.

Findings:

Recovery of radioactivity was approximately 92 % of the administered dose and appeared to be independent of dose level. Recovery data indicated that by 48 hours after dose administration, approximately 2.4 % or less remained in the carcass and the remainder either had been eliminated by the animal or was present in the GI tract plus contents, regardless of dose level.

Biliary excretion at the low dose averaged 33.90 % ± 3.97 %, at the high dose 25.03 % ± 7.21 % of the administered dose. Maximum levels of radiolabel in bile at the low dose occurred between 1 and 6 hours postdose (median time to peak 5 hours), at the high dose maximum levels occurred between 2 and 12 hours postdose (median time to peak between 7 and 10 hours). At the terminal collection (48 hours), an average of 0.13 % (low dose) and 0.12 % (high dose) of the administered dose was found in bile. However, by 24 hours, biliary excretion appeared to be 92 % complete at the low and 80 % at the high dose.

Urine was a minor excretory route. The recovery of radioactivity in urine of rats of the low dose group was 2.23 % ± 1.24 % of the administered dose. At the high dose, recovery was 1.21 % ± 0.78 % of the administered dose.

The mean recovery of radioactivity in the cage wash was 0.18 % ± 0.13 % at the low and 0.20 % ± 0.16 % at the high dose.

The amounts of radiolabel in carcasses 48 hours after dosing were 2.40 % ± 0.97 % at the low and 2.35 % ± 0.55 % at the high dose.

The amounts of radioactivity in blood represented only a fraction of a percent of the administered

dose at termination.

Absorption was calculated as the sum of radioactivity found in bile, urine, cage wash, carcass and blood. At the low dose, 38.86 % ± 4.69 %, at the high dose 28.89 % ± 7.21 % were absorbed. Biliary radioactivity accounted for approximately 87 % of the absorbed dose. More than half of the administered dose was not absorbed at either dose level. At the low dose, 48.44 % ± 8.76 % were detected in feces and 3.74 % ± 6.09 % in the GI tract plus contents. At the high dose, 61.51 % ± 11.91 % were found in feces and 2.98 % ± 3.95 % in the GI tract plus contents (table 6.1.1-6).

Table 6.1.1-6: Recovery of radioactivity in bile, urine, carcass, blood, cage wash, feces and GI tract (mean % of radioactive dose) after oral application of (14C)-fluazinam to rats

	Low dose (%)	High dose (%)
Bile	33.90	25.03
Urine	2.23	1.21
Carcass	2.40	2.35
Blood	0.14	0.10

	Low dose (%)	High dose (%)
Cage wash	0.18	0.20
Absorbed	38.86	28.89
Feces	48.44	61.51
GI tract	3.74	2.98
Total recovery	90.04	93.38

Conclusion:

At both dose levels, bile contained more than 86 % of the absorbed radiolabel. An average of 33.90 % of the administered dose is excreted in bile at the low dose and 25.03 % at the high dose. Carcasses accounted for less than 2.5 % and urine less than 2.25 % of the administered dose.

Feces together with the GI tract and contents represent the nonabsorbed dose and contained 52.18 % and 64.49 % for the low and high dose respectively.

Study to Identify the Metabolites of IKF-1216 (Fluazinam) in Rats

Reference: *McClanahan R. H. et al; 1995;* Report No. 5306-92-0191-AM-002

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation, Human and Domestic Animals, 85-1.

GLP: yes

Material and method:

Samples of feces, urine and bile were obtained from in-life rat metabolism studies *(Study to evaluate the distribution and excretion of (Phenyl-14C)-IKF-1216 14C(B)-IKF-1216 in rats, Report No. 5304-92-0185-AM-001; Study to evaluate the distribution and excretion of (Phenyl-14C)-IKF-1216 in rats following repeated dosing, Report No. 5317-93-0021-AM-001; Study of the biliary excretion of radiolabel following oral administration (Phenyl-14C)-IKF-1216 to male Sprague-Dawley rats, Report No. 5318-92-0321-AM-001* and an additional biliary metabolism study comparing the metabolism of ^{14}C-Phenyl (^{14}C)-fluazinam and ^{14}C-Pyridyl (^{14}C)-fluazinam, *Report No. 5306-92-0191-AM-002).* Samples were analyzed for parent compound and metabolites. Metabolites were identified using several techniques including HPLC coelution with standards, direct identification by mass spectrometry and comparison with standards, NMR, and degradation experiments. The distribution of these metabolites, as a function of dosing regimen, position of radiolabel, and sex, was determined.

Findings:

Major metabolites isolated and identified from feces, urine and bile were the parent compound, DAPA, AMPA, AMPA mercapturate, DAPA glucuronide and DAPA cysteine conjugate. The major metabolites of the organic fraction of feces were parent compound, AMPA and DAPA and the major metabolite in the aqueous fraction of feces was DAPA cysteine conjugate. The feces were the major route of excretion of fluazinam and its

metabolites. AMPA mercapturate, DAPA glucuronide and DAPA were found in the urine at low levels (≤ 2 % of administered dose) and AMPA mercapturate and DAPA glucuronide were found in the bile (≤ 5 % of administered dose). Fluazinam was also metabolized by the intestine microflora to form AMPA and DAPA. The identified metabolites were the same in samples from both phenyl and pyridyl labels, indicating that metabolic cleavage of the two rings did not occur. The metabolism of fluazinam was similar between male and female rats within a dose group. The chemical identity of each of these metabolites is given in table 6.1.1-7.

Table 6.1.1-7: Chemical identity of metabolites

Compound	Chemical name (IUPAC)
Fluazinam	3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- α,α,α-trifluoro-2,6-dinitro-p-toluidine
AMPA	2-(6-amino-3-chloro-α,α,α-trifluoro-2-nitro-p-toluidino)-3-chloro-5-(trifluoromethyl)pyridine
AMPA Mercapturate	N-acetyl-S-[4-amino-5-[[3-chloro-5-(trifluoromethyl)-2-pyridyl]amino]-α,α,α-trifluoro-6-nitro-o-tolyl]-L-cysteine
DAPA	3-chloro-2-(2,6-diamino-3-chloro-α,α,α-trifluoro-p-toluidino)-5-(trifluoromethyl)pyridine
DAPA Glucuronide (Isomer A)	1-[5-amino-4-chloro-6-[[3-chloro-5-(trifluoromethyl)-2-pyridyl]amino]- α,α,α-trifluoro-m-toluidino-1-deoxy-β-D-glucopyranuronic acid
DAPA Glucuronide (Isomer B)	1-[5-amino-2-chloro-6-[[3-chloro-5-(trifluoromethyl)-2-pyridyl]amino]- α,α,α-trifluoro-m-toluidino-1-deoxy-β-D-glucopyranuronic acid

Biliary metabolism studies: The overall accountability of identified metabolites in the biliary metabolism studies ranged from 63 – 71 % of the administered dose.
Fluazinam, AMPA and DAPA were identified as the major metabolites in the fecal extract.
AMPA mercapturate and DAPA glucuronide were identified as the major metabolites in the bile from animals dosed with either phenyl- or pyridyl-labeled fluazinam. These metabolites each comprised 5 % or less of the administered dose.
AMPA mercapturate, DAPA glucuronide and DAPA were identified as the major metabolites in urine and comprised each 2 % or less of the administered dose.
The identified metabolites were the same from animals dosed with either phenyl- or pyridyl-labelled fluazinam also, indicating that metabolic cleavage of the two rings did not occur.
Table 6.1.1-8 includes an extrapolated accountability for the 12 hour fecal samples which were not analyzed due to the fact that radioactivity was in very small quantities of feces that were used up in the mass balance measurements for the in-life study. The extrapolated accountability was based on data from analysis of the 24 hour fecal samples. No differences in overall accountability between sexes or label groups were observed.

Table 6.1.1-8: Accountability of identified metabolites from rat comparative biliary studies

		Percentage of Administered Dose			
Compound	Matrix	Phenyl	Phenyl	Pyridyl	Pyridyl
		Male	female	Male	female
Fluazinam	Feces	24.87	45.42	27.59	35.64
AMPA	Feces	3.57	5.69	4.09	5.07
DAPA	Feces	2.49	7.46	3.68	3.36
DAPA	Urine	0.07	0.18	0.48	0.43
DAPA Glucuronide	Urine	0.05	0.25	1.33	0.13
DAPA Glucuronide	Bile	3.98	2.14	1.47	2.86
AMPA Mercapturate	Urine	0.06	0.74	1.83	0.48
AMPA Mercapturate	Bile	3.81	2.71	3.12	0.87
Total		38.90	64.59	43.59	48.84
Extrapolated for 12 h fecal samples		23.92	0.00	24.65	22.26
Overall Total		62.82	64.59	68.24	71.10

Distribution studies: The overall accountability of identified metabolites in the distribution studies ranged from 11 – 15 % (low dose, 0.5 mg/kg bw ^{14}C-fluazinam) and 54 – 69 % (high dose, 50 mg/kg bw ^{14}C-fluazinam) of the administered dose.

Fluazinam, AMPA and DAPA were identified as the major metabolites in the fecal extract and accounted for 15.5 % (males) and 11.2 % (females) in the low dose group and for 53.5 % (males) and 68.2 % (females) in the high dose group.

AMPA mercapturate, DAPA glucuronide and DAPA were identified as the major metabolites in urine of the high dose groups. Urine was not analyzed for the low dose group due to the low levels of radioactivity.

AMPA mercapturate and DAPA glucuronide were excreted via the bile into the GI tract and were then metabolized to form DAPA and DAPA cysteine conjugate, which were identified as fecal metabolites. Unabsorbed fluazinam was also metabolized by the intestine microflora to form AMPA and DAPA.

The overall accountability of identified metabolites in the multiple dose study ranged from 34 – 42 % of the administered dose. Fluazinam, AMPA and DAPA were identified as the major metabolites in the fecal extract and accounted for 33.7 % (males) and 41.3 % (females) of the administered dose.

AMPA mercapturate and DAPA were identified as the major metabolites in urine and comprised less than 1 % of the administered dose.

Table 6.1.1-9: Total accountability of identified metabolites from rat distribution studies

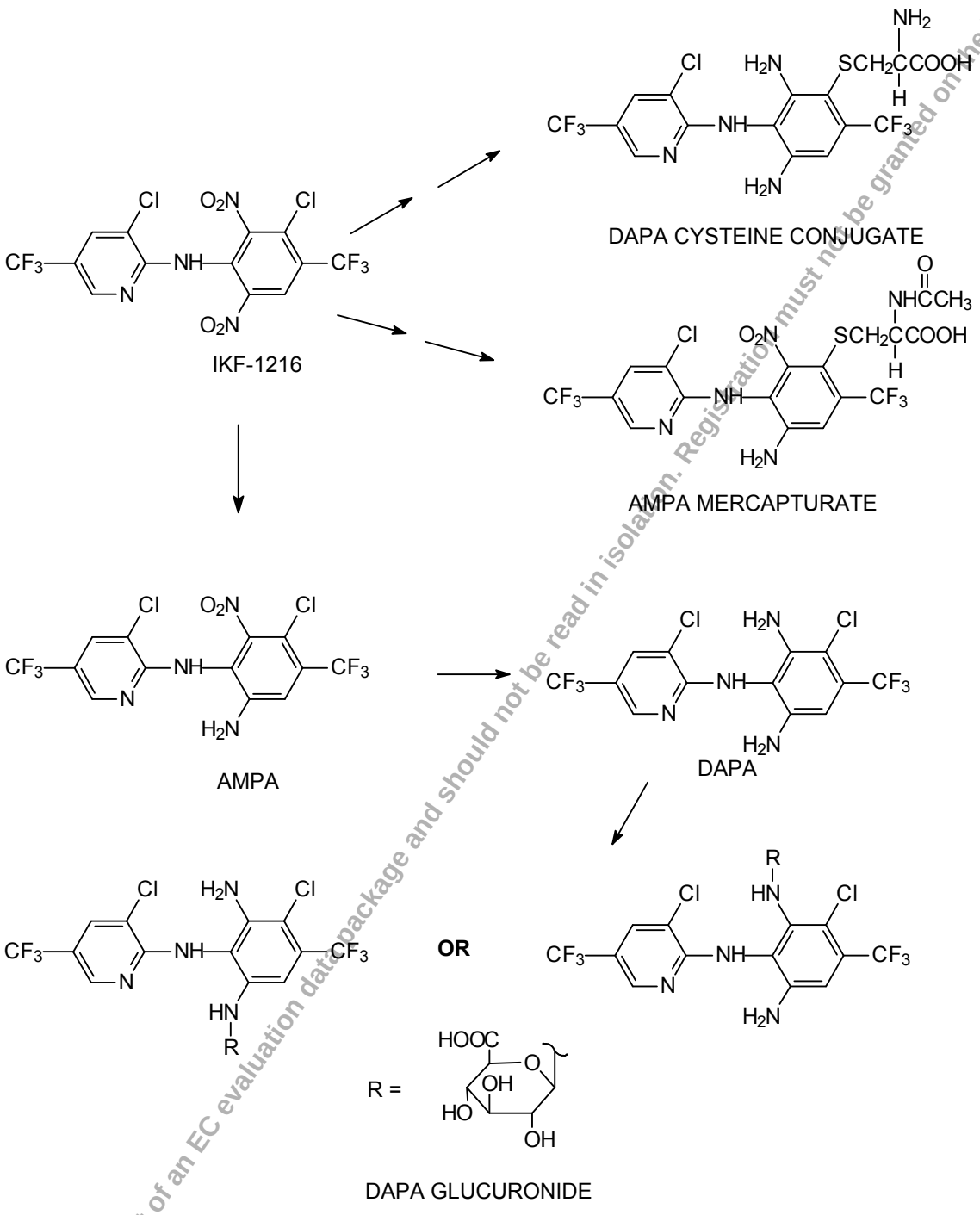
		Percentage of Administered Dose		
Compound	Matrix	High dose	Low dose	Multiple dose

		male	female	male	female	male	female
Fluazinam	Feces	45.13	54.93	7.64	2.13	27.47	36.83
AMPA	Feces	5.01	10.22	3.33	4.43	4.55	3.46
DAPA	Feces	3.36	3.07	4.52	4.64	1.72	0.99
DAPA	Urine	0.07	0.18	n.a.	n.a.	0.05	0.23
DAPA Glucuronide	Urine	0.00	0.00	n.a.	n.a.	n.a.	n.a.
AMPA Mercapturate	Urine	0.15	0.19	n.a.	n.a.	0.08	0.39
Total		53.72	68.59	15.49	11.20	33.87	41.90

n.a.: not analyzed due to the low levels of radioactivity

The metabolic pathway of fluazinam in rats involves reduction of one or both nitro groups (to form AMPA or DAPA), replacement of the phenyl ring chlorine by glutathione conjugation and then further metabolism/conjugation as shown below.

Figure 6.1-1: Metabolic Pathway of Fluazinam in Rats



Conclusion:

Fluazinam is metabolized in the liver to form AMPA mercapturate, DAPA glucuronide and DAPA. All of these metabolites were found as urinary metabolites. AMPA mercapturate and DAPA glucuronide were excreted into the bile. Fluazinam is also metabolized by the intestine microflora to form AMPA and DAPA. Unmetabolized fluazinam, AMPA and DAPA were identified as components of the feces from animals from the biliary metabolism studies. The

identified metabolites were the same in samples from both phenyl-or pyridyl-labels, indicating that metabolic cleavage did not occur.

B.6.1.2 Summary of ADME studies

Metabolic and kinetic studies were conducted with radiolabeled fluazinam, following oral administration at a low dose of 0.5 mg/kg bw, a high dose of 50 mg/kg bw and 14 daily oral doses of unlabeled fluazinam followed by ^{14}C-fluazinam (labelled in the phenyl position) of 0.5 mg/kg bw. The majority of radiolabeled material was detected in the feces (> 88 %). Urine was a minor excretory route (2 - 4 %). Less than 1 % of the administered dose was found in the carcass. The highest concentration was detected in the liver. There were no major differences related to sex or dose level. The median peak time for blood concentration of radiolabel activity for both sexes was 6 hours. At the time of peak concentration, the radioactivity in the blood represented 0.4 % - 0.6 % of the administered dose for 0.5 and 50 mg/kg bw dose groups. By 72 hours, about 0.1 % of the administered dose was found in the blood of both sexes at both dose levels. Approximately 30 % (high dose) – 40 % (low dose) of fluazinam was considered to be absorbed based on excretion rates in bile and urine. The predominant route of excretion of the absorbed dose was the bile, which contained approximately 87 % of the absorbed dose. 24 hours after dose administration, biliary excretion of the absorbed dose was 80 % complete at the high dose level and 92 % complete at the low dose level.

Metabolites were identified using several techniques including HPLC coelution with standards, direct identification by mass spectrometry and comparison with standards, NMR, and degradation experiments. The distribution of these metabolites, as a function of dosing regimen, position of radiolabel, and sex, was determined. Major metabolites isolated and identified from feces, urine and bile were the parent compound, DAPA, AMPA, AMPA mercapturate, DAPA glucuronide and DAPA cysteine conjugate. The major metabolites of the organic fraction of feces were parent compound, AMPA and DAPA and the major metabolite in the aqueous fraction of feces was DAPA cysteine conjugate. The feces were the major route of excretion of fluazinam and its metabolites. AMPA mercapturate, DAPA glucuronide and DAPA were found in the urine at low levels (≤ 2 % of administered dose) and AMPA mercapturate and DAPA glucuronide were found in the bile (≤ 5 % of administered dose). Fluazinam was also metabolized by the intestine microflora to form AMPA and DAPA. The identified metabolites were the same in samples from both phenyl and pyridyl labels, indicating that metabolic cleavage of the two rings did not occur. The metabolism of fluazinam was similar between male and female rats within a dose group. It can be concluded that fluazinam is metabolized by both reduction and glutathione conjugation and further metabolism.

B.6.2 Acute toxicity, irritancy and skin sensitization (Annex IIA 5.2)

B.6.2.1 Acute oral studies

Acute oral toxicity study in the mouse:

Reference: *Cummins, H.A.; 1988;* Report No. 87/ISK106/860

Guideline: The study was conducted according to OECD guideline 401 (1981), Japanese MAFF Test Guidelines (1985) and U.S. EPA Pesticide Assessment Guidelines (1982).

GLP: yes

Material and Methods:

Groups of 5 mice/sex (strain: CD-1; source: Charles River (U.K.) Limited, Margate, Kent) weighing between 17 and 27 g (5 weeks old) received a single oral dose of 5000 mg/kg bw fluazinam (batch no. 1/87; purity 97.9 %, suspended as a 50 % w/v formulation in maize oil) by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All mice were necropsied and macroscopic organ changes reported.

Findings:

Clinical signs and mortality:

There were no treatment-related deaths. Signs of reaction to treatment were confined to decreased motor activity 15 and 30 minutes after dosing. All animals were overtly normal thereafter.

Pathology:

No macroscopic organ changes were noted at necropsy.

Conclusion:

Under the conditions of this study, the acute oral toxicity of fluazinam (suspended as a 50 % w/v formulation in maize oil) in mice of both sexes observed for a period of 15 days was greater than 5000 mg/kg bw.

Acute oral toxicity study in the rat:

Reference: *Cummins, H.A.; 1988;* Report No. 87/ISK105/859

Guideline: The study was conducted according to OECD guideline 401 (1981), Japanese MAFF Test Guidelines (1985) and U.S. EPA Pesticide Assessment Guidelines (1982).

GLP: yes

Material and Methods:

Groups of 5 rats/sex (strain: Sprague-Dawley; source: Charles River (U.K.) Limited, Margate, Kent) weighing between 95 and 116 g (5 weeks old) received a single oral dose of 5000 mg/kg bw fluazinam (batch no. 1/87; purity 97.9 %, suspended as a 50 % w/v formulation in maize oil) by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All animals were necropsied and macroscopic organ

changes reported.

Findings:

Clinical signs and mortality:

One female and one male rat died on days 3 and 4 respectively. Signs of reaction to treatment were confined to decreased motor activity, hunched posture, piloerection, ungroomed condition and ataxia. Gasping or hairloss and pigmented staining of the snout were also observed. Surviving animals were fully recovered on day 5 and remained overtly normal during the 14-day observation period.

Pathology:

Necropsy of the two animals which died during the observation period revealed yellow mucus or dark viscous fluid in the gastrointestinal tract respectively. No macroscopic organ changes were noted for animals killed on day 15 of the study.

Conclusion:

Under the conditions of this study, the acute oral LD_{50} for fluazinam (suspended as a 50 % w/v formulation in maize oil) in rats of both sexes observed for a period of 15 days was greater than 5000 mg/kg bw.

Acute oral toxicity study in the rat:

Reference: *Liggett, M.P.; 1988;* Report No. 881246D/ISK20/AC

Guideline: The study was conducted according to Japanese MAFF Test Guidelines for Toxicology Studies, Acute Oral Toxicity Study and in accordance with 59 NohSan No. 4200, 1985.

GLP: yes

Material and Methods:

Groups of 5 rats/sex (strain: Sprague-Dawley; source: Interfauna U.K. Limited, Huntingdon, Cambridgeshire, England) weighing between 122 and 150 g (4 to 6 weeks old) received a single dose of either 2500, 3200, 4000 and 5000 mg/kg bw fluazinam (batch no. 109; purity 95.3 %, suspended in 1 % w/v aqueous methylcellulose) by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All animals were necropsied and macroscopic organ changes reported.

Findings:

Clinical signs and mortality:

Signs of reaction to treatment observed in all rats within five hours of dosing were piloerection, hunched posture, waddling, lethargy, pallor of the extremities and diarrhoea. Decreased respiratory rate and ptosis were observed at a concentration of 3200 mg/kg bw and above. Deaths occurred among both sexes from day 2 until day 4 at doses of 4000 mg/kg bw and above. Surviving animals were fully recovered at intervals from day 3 to day 5.

Pathology:

Necropsy revealed no macroscopic organ changes.

Conclusion:

Under the conditions of this study, the estimated acute oral LD_{50} for fluazinam (suspended in 1 % w/v aqueous methylcellulose) in rats observed for a period of 15 days was 4500 mg/kg bw for males and 4100 mg/kg bw for females (95 % confidence limits).

Table 6.2.1.1: Mortality induced by fluazinam in rats after oral exposure

Dose level	Mortality		
(mg/kg bw)	Males	Females	Total
2500	0/5	0/5	0/10
3200	0/5	0/5	0/10
4000	1/5	3/5	4/10
5000	4/5	4/5	8/10

B.6.2.2 Acute percutaneous studies

Acute percutaneous toxicity in the rat:

Reference: *Cummins, H.A.; 1984;* Report No. 84/ISK051/586

Guideline: The study was conducted according to OECD guideline 402 (1981).

GLP: yes

Material and Methods:

Groups of 5 rats/sex (strain: Sprague-Dawley (CD); source: Charles River (U.K.) Limited) weighing between 200 and 246 g (10 weeks old) received a single dose of 2000 mg/kg bw fluazinam (batch no. 8303-2; purity 98.5 %). The dose was applied as a thin layer covering the shaven dorsum (dorsum was moistened by application of 0.2 ml distilled water to intend good contact between test material and skin) and was covered by an unmedicated gauze dressing and aluminium foil. The dressings were removed 24 hours after administration. The dermal site was brushed free of the test material and wiped with wet disposable towels. Each test site was inspected daily for dermal reactions to treatment. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All animals were necropsied and macroscopic organ changes reported.

Findings:

There were no deaths and no reaction to treatment. There was no evidence of local irritation at the site of application. All animals made anticipated bodyweight gains during the 14-day observation period and necropsy on day 15 revealed no macroscopic abnormality.

Conclusion:

Under the conditions of this study, the acute percutaneous LD_{50} of fluazinam in rats of both sexes observed for a period of 15 days was greater than 2000 mg/kg bw.

B.6.2.3 Acute inhalation study

Acute inhalation toxicity test of fluazinam technical in rats:

Reference: *Tobeta, Y.; 1988;* Report No. D/1775E

Guideline: The study was conducted according to Japanese MAFF Test Guidelines for Toxicology Studies (NohSan No. 4200, 59) and U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-3 (1984).

GLP: yes

Material and Methods:

Groups of 10 rats/sex (strain: Sprague-Dawley (SPF); source: Charles River Japan) weighing between 146 and 227 g (6 weeks old) were exposed for four hours (whole body exposure) to an atmosphere containing a 10 % solution of fluazinam (batch no. 109; purity 95.3 %) in polyethylene glycol 400 at concentrations of 0.309, 0.407, 0.532 and 0.684 mg a.i./l in air. Polyethylene glycol 400 was used as solvent control. Animals were exposed in a stainless steel inhalation chamber of approximately 380 l capacity. The mass median aerodynamic diameter (MMAD) of the aerosol particles ranged from 3.0 $\pm$ 1.82 µm to 3.53 $\pm$ 1.86 µm. Animals were observed for clinical signs during exposure and 10, 30, 60 and 120 minutes after its termination. Thereafter they were observed twice daily for 14 days. Body weights were recorded immediately before exposure (day 0) as well as 3, 5, 7, 10 and 14 days after exposure. At the end of the 14-day observation period, all surviving rats were exsanguinated and necropsied. Animals dying during the study were necropsied immediately after death was noted.

Findings:

Clinical signs and mortality: During exposure, all animals showed reduced spontaneous movement, moist fur, nasal blot, cloudy eyeballs, decreased respiratory rate and gasping or abnormal breathing sound.

Mortality occurred in males within 7 days after exposure and in females within 4 days after exposure.

Pathology: Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered mostly due to respiratory failure. Necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities (Table 6.2.3.1).

Table 6.2.3.1: Mortality induced by fluazinam in rats after a four-hour inhalation (whole body exposure)

Sex	Actual Concentration	Day								
		0	1	2	3	4	5	6	7-14	Fin. Mort
Male	0.684	1	2	0	0	1	1	0	0	5/10
	0.532	3	3	0	0	0	0	1	0	7/10
	0.407	1	3	0	0	0	0	0	0	4/10

Sex	Actual Concentration	Day								
		0	1	2	3	4	5	6	7-14	Fin. Mort
	0.309	1	3	0	0	0	0	0	0	4/10
	Solvent Control	0	0	0	0	0	0	0	0	0/10
Female	0.684	4	4	0	1	0	0	0	0	9/10
	0.532	2	3	0	0	0	0	0	0	5/10
	0.407	1	3	0	0	0	0	0	0	4/10
	0.309	1	0	0	0	0	0	0	0	1/10
	Solvent Control	0	0	0	0	0	0	0	0	0/10

Conclusion:

The acute inhalation LC_{50} (4 hour exposure) for fluazinam was 0.463 mg/l for males and 0.476 mg/l for females. However, given the conditions of the study, a mixed oral, dermal and inhalative exposure cannot be excluded.

B.6.2.4 Skin irritation

Primary dermal irritation study in albino rabbits:

Reference: *Shults, S. K.; 1992;* Report No. 5016-91-0281-TX-001

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-5.

GLP: yes

Material and Methods:

The back of 3 male and 3 female New Zealand White rabbits (source: Mohican Valley Rabbitry, Loudonville, Ohio, resp., weighing between 2128 and 2429 g) was clipped free of hair with electric clippers. Each rabbit received 0.5 g Fluazinam Technical (batch no. 1006; purity 97.9 %; moistened with deionized water) at an approximately one inch square dorsal skin site. The test site was dressed with an occlusive wrap for an exposure period of 4 hours. Following the exposure period, the test sites were wiped with paper towels (wetted with water) and examined for local skin reactions and scored and evaluated for erythema, eschar and edema using the method of Draize (1959). Reading of the individual scores is reported within 30 to 60 minutes and then at approximately 24, 48 and 72 hours following removal of the patch and on days 4 through 13 of the study. During the study, all animals were observed twice daily for mortality and moribundity also.

Findings:

Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study.

Mean irritation scores are given in table 6.2.4.1. Slight to well defined erythema was observed in all 6 rabbits at the 30 and 60 minute interval and in 5 rabbits at the 24 and 48

hour intervals. On day 4, erythema was observed in 4, on day 5 in 3 animals and persisted till day 11 in 2 animals and in one rabbit till day 12. No edema was observed in any of the rabbits during the study. The primary irritation index for erythema was calculated to be 0.9.

Table 6.2.4.1: Individual and mean skin irritation scores in albino rabbits with fluazinam technical

Animal	Erythema													
	Min	Hour			Days									
	30-60	24	48	72	4	5	6	7	8	9	10	11	12	13
M1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
M2	1	1	1	0	0	0	0	0	0	0	0	0	0	0
M3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F1	1	1	1	2	2	2	2	2	2	1	1	1	1	0
F2	1	1	1	1	1	1	0	0	0	0	0	0	0	0
F3	1	1	1	1	1	0	0	0	0	0	0	0	0	0
Mean	1.0	0.8	0.8	0.8	0.8	0.7	0.5	0.5	0.5	0.3	0.3	0.3	0.2	0.0

M = Male rabbit, F = Female rabbit

Conclusion:

Given the mean irritation scores at 24, 48 and 72 hours, fluazinam can be considered as a slight irritant using the Draize criteria for evaluation.

B.6.2.5 Eye irritation

Primary eye irritation study in albino rabbits:

Reference: *Shults, S. K.; 1992;* Report No. 5016-91-0280-TX-002

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-4.

GLP: yes

Material and Methods:

Six adult New Zealand White rabbits (3 males, 3 females; source: Mohican Valley Rabbitry, Loudonville, Ohio), weighing between 2122 and 2729 g, received a single application of 0.1 g Fluazinam Technical (batch no. 1006; purity 97.9 %;) into the conjunctival sac of the right eye. The eyelids were held together for one second following instillation. The left eyes remained untreated and served as a control. The treated eyes remained unwashed. Treated and control eyes were examined for signs of irritation at 1, 24, 48, 72 hours and on days 4, 7, 10, 14 and 21 after dosing. Fluorescein sodium ophthalmic solution and an ultraviolet lamp were used to aid in ocular examinations at 72 hours after treatment and on days 7, 14 and 21 postdose. After completion of eye examination on day 21 the study was terminated and all animals sacrificed without further examination. Grading and scoring of the ocular lesions were performed in accordance with the Draize system.

Findings:

Corneal opacity was observed in treated eyes of all six rabbits at the 24 and 48 hour intervals and in five rabbits at the 72 hour interval and on day 4. In one animal corneal opacity persisted till termination of the study on day 21. Corneal vascularisation involving up to approximately 5 % of the cornea was observed in one rabbit on day 4 and in 2 rabbits on day 7. In one rabbit vascularisation persisted till termination of the study. Using fluorescein sodium ophthalmic solution indicated significant corneal epithelial effects involving up to approximately 25 % of the corneal surface in 3 rabbits at 72 hours and persisted in 2 animals through day 7 of the study.

Iridal effects were observed in 4 rabbits and persisted in one animal till termination on day 21. Conjunctival irritation was observed in all six rabbits at the 1 hour interval and persisted in one animal till day 21. Mean scores calculated according to the Draize method, are given in table 6.2.5.1.

Table 6.2.5.1: Individual eye irritation scores

Rabbit No.	Time after treatment	Corneal opacity	Iridial inflammation	Conjunctival redness	Conjunctival chemosis
202727	1 hour	0	0	2	2
	24 hours 48 hours 72 hours	2 2 2	1 1 1	3 3 3	4 3 2
	Mean 24 – 72 h	2	1	3	3
202728	1 hour	0	0	1	1
	24 hours 48 hours 72 hours	2 3 3	1 1 1	3 3 3	3 3 2
	Mean 24 – 72 h	2.6	1	3	2.6
202729	1 hour	0	0	1	1
	24 hours 48 hours 72 hours	2 1 0	0 0 0	3 2 1	3 1 1
	Mean 24 – 72 h	1	0	2	1.6
202730	1 hour	0	0	2	1
	24 hours 48 hours 72 hours	1 1 1	1 0 0	3 2 1	3 1 1
	Mean 24 – 72 h	1	0.3	2	1.6
202731	1 hour	0	0	2	1
	24 hours 48 hours 72 hours	1 1 1	1 0 0	3 3 2	3 2 1
	Mean 24 – 72 h	1	0.3	2.6	2
202732	1 hour	0	0	2	2
	24 hours 48 hours 72 hours	1 1 1	0 0 0	3 3 2	3 2 1
	Mean 24 – 72 h	1	0	2.6	2

Conclusion:

Fluazinam produced corneal, iridal and conjunctival effects which persisted partly through day 21 of the study. So fluazinam has to be considered an severe eye irritant.

B.6.2.6 Skin sensitization

Delayed contact hypersensitivity study in guinea-pigs:

Reference: *Cummins, H.A.; 1984;* Report No. 84/ISK054/686

Guideline: The study was performed in accordance with U.S. EPA Pesticide Assessment Guidelines Subdivision F, No. 81-6 (Magnusson and Kligman).

GLP: yes

Material and Methods:

20 guinea pigs (10 males, 10 females; strain: Dunkin-Hartley; source: Olac 1976 Ltd., Bicester, Oxfordshire), received fluazinam (batch no. 8303-2; purity 98.5 %) intradermally and topically. Additionally, 10 male and 10 female guinea pigs were used as negative control group and 5 males and 5 females served as positive controls. The concentrations used for the treatment in this study were based on the results of a preliminary skin irritation screening study.

In the main study, intradermal induction (three pairs of injections, 0.1 ml/injection) was performed with Freunds Complete Adjuvant (anterior sites), 10 % w/v solution of fluazinam in paraffin oil (middle sites) and 10 % w/v solution of fluazinam in Freunds Complete Adjuvant (posterior sites) by intradermal injections into the dermis on either side of the dorsal median line parallel to the spinal column at the scapular region. Control animals received similar injections except fluazinam was replaced by paraffin oil. Dinitrochlorobenzene was used for positive control group (0.6 % w/v DNCB in paraffin oil: induction and challenge). The day of intradermal induction was designated day 1.

Dermal responses to primary induction were assessed 24 and 48 hours after administration. Topical induction (for 48 hours under occlusive dressing at the injection test sites) was carried out on day 8 using a concentration of 0.4 ml 70 % (w/v) fluazinam in paraffin oil. Paraffin oil was used in replacement of fluazinam for the control group. Dermal responses to secondary induction were assessed 24 and 48 hours after removal of the occlusive dressing. On day 22 the challenge phase was performed in the treated group and in the control group by applying 0.2 ml 70 % (w/v) solution of fluazinam in paraffin oil dermally under occlusive dressing for 24 hours on the right flank (50 x 50 mm area) while the left flank received the vehicle only. The dressings were removed 24 hours later and skin reactions were quantified 4, 24 and 48 hours thereafter macroscopically.

Findings:

Primary induction: Signs of irritation (erythema) were noted during induction after intradermal injection of formulations containing fluazinam and/or Freunds Complete Adjuvant. Sites treated with fluazinam frequently became discoloured. Control group animals showed no dermal response.

Topical induction: Two animals showed slight to moderate erythema 24 hours after removal of the occlusive dressings which applied 70 % w/v fluazinam in paraffin oil to the shaven dorsum. After 48 hours dermal response was neither seen in the test group animals nor in the control group.

Challenge:

70 % (w/v) solution of fluazinam in paraffin oil (right flank): 4 hours after removal of the occlusive dressing all animals showed slight to moderate erythema. 24 hours after completion of challenge 5 animals from each group showed slight, one animal of the test group showed moderate erythema. After 48 hours, slight erythema was observed in one control and in 2 test group animals. 3 test group animals showed exfoliation of the right flank

challenge site.

Paraffin oil (left flank): After challenge, 6 control and 13 test group animals had developed slight to moderate erythema of the treated skin at the first examination. After 24 and 48 hours no erythematous response was observed with the exception of one test group animal, which showed exfoliation.

Positiv control group animals showed dermal sensitization responses as expected.

Conclusion:

Based on the results of the study, fluazinam caused delayed contact hypersensitivity in guinea pigs.

Skin sensitisation to the guinea-pig of both the purified and technical material:

Reference: *Pritchard, V.A.; 1986;* Report No. CTL/P/1493

Guideline: The study was assessed by the sensitisation method developed by Buehler (1965) and in accordance to U.S. EPA Pesticide Assessment Guidelines Subdivision F, No. 81-6.

GLP: yes

Material and Methods:

Technical fluazinam (batch no. 5903-2 and 8412-20; purity 95.3 %) and purified fluazinam (batch no. 8505-1; purity 99.7 %) were used in this study.

Induction phase: 20 male guinea pigs (strain: Dunkin Hartley; source: Animal Breeding Unit, Imperial Chemical Industries PLC, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, UK), were treated topically with 0.4 ml of a 50 % w/v solution of fluazinam technical (batch no. 5903-2) in 0.5 % polysorbate 80. 10 male guinea pigs of the same strain served as controls and received 0.5 % polysorbate 80 only. Patches were applied onto the shaved left shoulder (50 mm x 50 mm) of the animals and removed after approximately 6 hours. These treatments were performed once a week, for three consecutive weeks. Following each induction, test sites were scored for dermal irritation 24 hours after removal of each patch and before application of each subsequent patch. Following the final induction application, animals were left untreated for a period of 14 days (rest phase). The concentration used for the treatment in this study was based on the results of a preliminary screening study and was the highest concentration which did not cause any irritation following a single application. Data of a positive control group are not reported.

For the challenge phase, flanks of the animals were shaved (150 mm x 50 mm). An occlusive dressing was prepared which consisted of 2 lint pads stitched to a piece of rubber sheeting. One lint pad (10 mm x 20 mm) containing 0.2 ml of a 50 % w/v suspension of fluazinam technical (batch no. 5903-2) in 0.5 % polysorbate 80 was applied on the right flank and the second lint pad containing 0.2 ml of a 50 % w/v suspension of purified fluazinam in 0.5 % polysorbate 80 was applied on the left flank. Test sides were occluded for 6 hours. At approximately 24 hours after patch removal, test sites were graded for dermal irritation (24-hour scoring period) and additionally after further 24 hours (48-hour scoring period).

14 days after the initial challenge, test animals were given a further topical induction of a 50 % w/v suspension of fluazinam technical (batch no. 5903-2). Seven days after the second induction animals were rechallenged using 50 % (w/v) preparations of both technical (batch no. 8412-20) and purified fluazinam in 0.5 % polysorbate 80. Both flanks were clipped free of hair and fluazinam was applied to different sites than those used for the initial challenge. A fresh group of ten male control animals was used for the rechallenge.

Findings:

Signs of moderate skin irritation (erythema, desquamation, thickening, edema and scabbing) were seen after the second and third inductions. Nine of 20 test animals and one of 10 controls had scattered mild or moderate and diffuse redness after challenge with the technical material. The net percentage response was 35% and, therefore, a 50% preparation of technical fluazinam elicited a moderate sensitization response in previously induced guinea pigs.

Three of 20 test animals and one of 10 controls had scattered mild redness after rechallenge with purified fluazinam. The net percentage response was 5% and, therefore, a 50% preparation of purified fluazinam elicited a weak sensitization response in previously induced guinea pigs.

Conclusion:

Using the sensitization method of Buehler, guinea pigs challenged with a 50% preparation of technical fluazinam and purified fluazinam elicited a moderate or weak sensitization response, respectively. When rechallenged, previously induced animals elicited a moderate sensitization response with a 50% (w/v) preparation of the technical material and a mild sensitization and an irritant response with the 50% (w/v) preparation of the purified fluazinam.

B.6.2.7 Summary of acute toxicity studies

After oral application to mice and rats of both sex, fluazinam is of low acute toxicity with LD_{50} values $\geq$ 4100 mg/kg bw.

After acute dermal application of fluazinam to rats of both sex, the acute dermal LD_{50} was > 2000 mg/kg bw. The inhalative LC_{50} of fluazinam in rats (whole-body exposure) was 0.46 mg/l.

Fluazinam is mildly irritating to the skin and severely irritating to the eyes of New Zealand White rabbits. In the Magnusson and Kligman dermal maximization study and in the Buehler-Test fluazinam caused evidence of delayed contact hypersensitivity in guinea pigs. A summary of the results from the acute toxicity studies is presented in table 6.2.7-1.

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as toxic by inhalation, severely irritating to the eyes (risk of serious damage to eyes) and as a sensitizer (hazard symbols T, Xi, risk phrases R 23, 41, 43).

Table 6.2.7-1 Summarised results of the acute toxicity studies with fluazinam

Type of study	Species	Vehicle	Results	Reference
Acute oral toxicity	CD-1 mice	Maize oil	m/f > 5000 mg/kg bw	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley - Rat	Maize oil	m/f > 5000 mg/kg bw	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat	Methylcellulose	m 4500 mg/ kg bw f 4100 mg/ kg bw	*Liggett, 1988*
Acute dermal toxicity	Sprague Dawley Rat	-	m/f > 2000 mg/kg bw	*Cummins, 1988*
Acute inhalation toxicity	Sprague Dawley Rat	Polyethylen glycol 400	m 0.463mg/l air f 0.476 mg/l air (4h, whole body exposure)	*Tobeta, 1988*
Dermal irritation study	Rabbit (NZW)	Moistened with deionized water	Mildly irritating	*Shults, 1992*
Eye irritation study	Rabbit (NZW)	-	severely irritating	*Shults, 1992*
Dermal sensitization M & K-test	Guinea pig (Dunkin Hartley)	Paraffin oil	Sensitizing	*Cummins, 1984*
Dermal sensitization Buehler –test	Guinea pig (Dunkin Hartley)	Polysorbate 80	Sensitizing	*Pritchard, 1986*

B.6.3 Short term toxicity (Annex IIA 5.3)

B.6.3.1 Oral studies in rats

4-Week Toxicity Study in Dietary Administration to CD Rats:

Reference: *Broadmeadow A. et al; 1983;* Report No. 82/ISK035/544

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is acceptable as supplementary information.

GLP: The study is in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060), specific GLP standards not indicated.

Material and method:

Groups of 10 male and 10 female rats (strain: CD rats (remote Sprague-Dawley); source: Charles River U.K., Margate, England) received diets containing 0, 10, 50, 250 and 3000 ppm fluazinam (batch 8203, purity 96.3 %), equivalent to 0, 1.26, 5.21, 26.1 and 305.4 mg/kg bw for 4 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed twice daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption was measured weekly, body weights were assessed on the first day of treatment, at twice weekly intervals thereafter and on the day of necropsy. Ophthalmoscopic examinations were done before initiation and after treatment (Pupils were dilated with 0.5 % tropicamide, then the eyes were examined with an

indirect ophthalmoscope). Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC differential), clinical chemistry (glucose, urea nitrogen, total protein, albumin, globulin, total bilirubin, cholesterol, alkaline aminophosphatase (AP), alanine aminotransferase (ALT), phospholipids, sodium, potassium, chloride) and urinanalysis (appearance, volume, pH, specific gravity, glucose, bilirubin, protein, ketones, blood, urobilinogen, sediment) parameters were evaluated for all surviving animals after 3 weeks of treatment. A necropsy was done on each animal that died or was sacrificed in a moribund condition and on all surviving animals after 4 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thyroid with parathyroid) were recorded. Histopathological examinations were performed on brain, heart, kidneys, marrow smear, liver, lungs with main stem bronchi, spleen and testes.

Findings:

General observations: There were no clinical signs, treatment-related changes in behaviour, locomotor function or gross necropsy observations.

Food consumption and body weight gains of males and females receiving 3000 ppm and females receiving 250 ppm were significantly lower than those of the respective control values.

Ophthalmoscopic examinations revealed no evidence of treatment-related changes at any dose group.

Evaluation of hematological parameters revealed significantly lower haemoglobin (Hb) concentrations in males and females given 3000 ppm and lower platelet counts in females of this dosing group. There were no other notable or statistically significant differences for hematology.

Concerning clinical chemistry, low alanine amino transferase activity in both sexes given 3000 ppm and in males receiving 250 ppm were observed. Blood cholesterol concentrations of both sexes at dose levels of 3000 and 250 ppm and phospholipid concentrations of males and females receiving 3000 ppm and females receiving 250 ppm were also significantly higher compared to controls.

(table 6.3.1.-1)

Table 6.3.1-1: Relevant haematological and clinical chemistry findings (group mean values) in rats after 4 weeks of treatment with fluazinam

Parameter	Dose group level (ppm)									
	Males					Females				
	0	10	50	250	3000	0	10	50	250	3000
Hemoglobin (g%)	15.2	14.6[a]	14.5[b]	14.4[b]	14.2[c]	14.3	14.4	14.7	14.8	13.1[b]
Platelet count (1000/cm^3)	785	830	837	791	741	700	777	743	724	611[a]

Parameter	Dose group level (ppm)									
	Males					Females				
	0	10	50	250	3000	0	10	50	250	3000
ALT (iu/l)	35	35	38	29[b]	25[c]	34	30	30	30	27[b]
Cholesterol (mg%)	51	50	54	60[a]	69[c]	53	61	57	66[b]	78[c]
Phospholipid (mg%)	153	154	161	173	186[b]	144	157	155	168[a]	201[c]

a: significantly different from controls at p<0.05; b: significantly different from controls at p<0.01; c: significantly different from controls at p<0.001 (Student's t-test)

Urinanalysis: there was no evidence of treatment-related changes at any dose group when compared to control animals.

Absolute organ weight analysis after 4 weeks of treatment revealed higher liver weights in males and females receiving 3000 ppm compared to controls. When corrected for bodyweight the liver weights of both sexes receiving 250 ppm and of males receiving 50 ppm were also higher than controls. Absolute ovary weights of females receiving 3000 ppm were lower compared to controls. (table 6.3.1-2).

Table 6.3.1-2: Body weights (g), absolute organ weights (g) and relative organ weights (expressed as a percentage) of rats after 4 weeks of treatment with fluazinam (group mean values)

	Dose group level (ppm)									
	Males					Females				
	0	10	50	250	3000	0	10	50	250	3000
Body weight	342	319	326	340	309[b]	215	208	203	203	189[b]
Liver (abs.)	17.2	16.5	18.0	18.5	19.7[b]	10.0	9.7	9.9	10.9	12.3[c]
Liver (rel.)	5.0	5.2	5.5[a]	5.4[a]	6.4[c]	4.6	4.6	4.9	5.4[c]	6.5[c]
Ovary (abs.)	NA	NA	NA	NA	NA	0.085	0.076	0.076	0.081	0.072[a]
Ovary (rel.)	NA	NA	NA	NA	NA	0.039	0.037	0.038	0.040	0.038

a: significantly different from controls at p<0.05; b: significantly different from controls at p<0.01; c: significantly different from controls at p<0.001 (Student's t-test)
NA = Not available

At necropsy, macroscopic examinations revealed no treatment-related changes.

Histopathological examinations revealed a higher incidence of single cell necrosis with mononuclear infiltration in the liver among females receiving 3000 ppm and a slightly higher incidence of periacinar hypertrophy among males receiving 250 or 3000 ppm (table 6.3.1-3).

Table 6.3.1-3: Incidences of microscopic findings of rats after 4 weeks of treatment with fluazinam

	0		10		50		250		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
single cell necrosis with mononuclear infiltration	0/10	0/10	2/10	0/10	3/10	2/10	1/10	0/10	1/10	6/10
Periacinar hypertrophy	1/10	0/10	0/10	0/10	0/10	0/10	3/10	0/10	5/10	0/10

Conclusion:

Dietary administration of fluazinam for 4 weeks caused higher relative liver weights in males and females receiving 250 ppm and also in males receiving 50 ppm. Histopathological examination revealed a higher incidence of periacinar hypertrophy among males receiving 250 or 3000 ppm. The NOAEL for fluazinam fed to rats for 4 weeks was 10 ppm, 1.26 mg/kg bw/d for males and females.

B-1216: 13-Week Liver Toxicity and 4-week Reversibility Study in Dietary Administration to CD Rats:

Reference: *Broadmeadow A. et al; 1985;* Report No. 84/ISK045/581

Guideline: No specific test guideline is mentioned in the study.

The aim of this study was to assess the hepatotoxic effects of fluazinam and to determine the reversibility of any such effects. Due to the restricted extent of parameters investigated, the study is considered as supplementary information.

GLP: The study is in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060).

Material and method:

One group of 20 male and 20 female rats (strain: CD rats (remote Sprague-Dawley); source: Charles River U.K., Margate, Kent, England) received a diet containing 500 ppm fluazinam (batch 8303-2, purity 98.5 %). The achieved intake ranged from 75 – 25 mg/kg bw (males, weeks 1 – 13) and 73 – 35 mg/kg bw (females, weeks 1 – 13), mean intake 40 mg/kg bw/d. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. A group of 20 male and 20 female rats served as controls, receiving untreated diet. Ten males and 10 females from each group were maintained for an additional four weeks, without treatment, in order to assess the reversibility of any treatment-related effect.

Animals were observed twice daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption was measured weekly, body weights were assessed on the first day of treatment, at weekly intervals thereafter and on the day of necropsy. At termination, the liver weight was recorded and the liver aminopyrine-N-demethylase activity was determined for all animals. A necropsy was done on each animal

and the liver was examined microscopically.

Findings:

General observations: There were no clinical signs and no animals died during the study.

Food consumption and food efficiency were unaffected by treatment. A marginally reduced body weight gain in treated females from week 4 on was considered to represent a mild, non-specific response to treatment. Body weights in treated males throughout the study and in treated females after the 4 week recovery period were similar to controls. There was no evidence of hepatic enzyme activity based on aminopyrine-N-demethylase activity measurements.

Relative liver weights of animals receiving 500 ppm fluazinam were significantly higher after 13 weeks of treatment. Absolute liver weights were not different from controls. After the 4 week recovery period relative liver weights were also similar to controls.

Histopathological examinations revealed a higher incidence of periacinar hepatocytic hypertrophy among males receiving 500 ppm. No similar change was seen in females or in formerly treated animals of either sex killed after 4 weeks of the recovery period.

Conclusion:

Dietary administration of fluazinam for 13 weeks at a concentration of 500 ppm caused higher relative liver weights in males and females and a higher incidence of periacinar hypertrophy among males. 4 weeks after treatment stopped, the reversal of the overall effects on the liver was almost complete.

13-Week Toxicity Study in Dietary Administration to CD Rats:

Reference: *Broadmeadow A. et al; 1984;* Report No. 84/ISK046/635, Amended final report No. 91/ISK046/0830 and Addendum 3 (1998)

Guideline: The study was conducted according to U.S. EPA Guideline 82-1 and is in compliance with GLP. The study is considered acceptable.

Material and method:

Groups of 10 male and 10 female rats (strain: CD rats (remote Sprague-Dawley); source: Charles River U.K., Margate, Kent, England) received diets containing 0, 2, 10, 50 and 500 ppm fluazinam (batch 8303-2, purity 98.5 %), equivalent to 0, 0.16, 0.82, 4.1 and 41 mg/kg bw for 13 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed twice daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption was measured weekly, body weights were assessed on the first day of treatment, at weekly intervals thereafter and on the day of necropsy. Ophthalmoscopic examinations were done before initiation and after treatment (Pupils were dilated with 0.5 % tropicamide, then the eyes were examined with an indirect ophthalmoscope). Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC differential), clinical chemistry (glucose, urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT,

AST, phospholipids, sodium, potassium, chloride, calcium, phosphor) and urinanalysis (appearance, volume, pH, specific gravity, glucose, bilirubin, protein, ketones, blood, urobilinogen, sediment) parameters were evaluated for all surviving animals after 6 and 12 weeks of treatment. A necropsy was done on each animal after 13 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid with parathyroid, uterus) were recorded. Liver aminopyrine-N-demethylase activity was determined for all animals. Histopathological examinations were performed on kidneys, liver, caecum and lungs of all animals. From all rats of the control and 500 ppm-groups histopathological examinations were also made on adrenals, brain, gastrointestinal tract, epididymides, eye and optic nerve, heart, lungs, marrow smear, lymph nodes, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spleen, sternum, testes, thymus, thyroid with parathyroid, urinary bladder, uterine cervix and uterus.
!n addition, a microscopic reevaluation of brain tissue and cervical spinal cord from all control and high dose level rats was conducted (Addendum 3, 1998). Slides were reviewed by the study pathologist and then subjected to a peer review by a second pathologist:

Findings:

General observations: There were no clinical signs or gross necropsy observations.
Food consumption was similar in treated and control animals.
Body weight gains of males and females receiving 500 ppm were marginally lower than those of the respective control values, but significance was not reached.
Ophthalmoscopic examinations revealed no evidence of treatment-related changes at any dose group.
Evaluation of hematological parameters revealed significantly lower hemoglobin concentrations, erythrocyte counts and packed cell volume in males receiving 500 ppm.
Blood chemistry examination did not reveal any changes compared to controls.
Urinanalysis: there was no evidence of treatment-related changes at any dose group when compared to control animals.
Liver function: There was no evidence of hepatic enzyme activity based on aminopyrine-N-demethylase activity measurements.
Organ weight analyses: Statistically significant higher relative liver weights were observed in males receiving 500 ppm compared to controls. Females showed an increase in absolute and relative lung and uterus weights at the highest dose level. Absolute lung weights of females receiving 2 and 500 ppm were statistically significantly higher compared to controls. Since there did not appear to be a dosage relationship, this was considered to be an equivocal effect (table 6.3.1-4).

Table 6.3.1-4: Body weights (g), absolute organ weights (g) and relative organ weights (expressed as a percentage) of rats after 13 weeks of treatment with fluazinam (group mean values)

	Dose group level (ppm)									
	Males					Females				
	0	2	10	50	500	0	2	10	50	500
Body weight	504	525	497	520	491	278	291	277	269	264
Liver (abs.)	18.3	20.0	18.4	20.4	19.7	10.9	11.2	11.1	10.4	11.1
Liver (rel.)	3.62	3.81	3.68	3.91	4.01*	3.93	3.86	4.03	3.89	4.22
Lungs (abs.)	1.96	2.01	1.94	2.09	1.81	1.34	1.60*	1.45	1.50	1.58*
Lungs (rel.)	0.389	0.382	0.396	0.403	0.369	0.481	0.550	0.527	0.562	0.599**
Uterus (abs.)	-	-	-	-	-	0.58	0.63	0.57	0.54	0.79*
Uterus (rel.)	-	-	-	-	-	0.209	0.215	0.208	0.203	0.298*

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01 (Dunnett's Test)

At necropsy, macroscopic examinations revealed no treatment-related changes. Histopathological examinations revealed a statistically significant higher incidence of periacinar hepatocytic hypertrophy among males receiving 500 ppm, compared with sinusoidal chronic inflammation. Chronic pneumonitis was found in lungs of females receiving 500 ppm. There was no effect of treatment on the incidence or degree of vacuolation of white matter in the brain or spinal cord of rats of the high dose group (500 ppm) compared to controls (table 6.3.1-5).

Table 6.3.1-5: Incidences of microscopic findings of rats after 13 weeks of treatment with fluazinam

	0		2		10		50		500	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
periacinar hypertrophy	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	8/10**	0/10
sinusoidal chronic inflammation	4/10	7/10	6/10	4/10	2/10	5/10	6/10	6/10	10/10*	8/10
Pneumonitis	5/10	3/10	5/10	6/10	6/10	6/10	9/10	7/10	6/10	10/10*

*: significantly different from controls at p<0.01; **: significantly different from controls at p<0.001 (Fisher's Exact Test)

Conclusion:

Dietary administration of fluazinam at a concentration of 500 ppm for 13 weeks caused marginally reduced body weight gain in both sexes. Liver weights were statistically significantly increased in males at a concentration of 500 ppm. Histopathological

examination revealed an incidence of periacinar hypertrophy among males receiving 500 ppm. The NOAEL for fluazinam fed to rats for 13 weeks was 50 ppm, 4.1 mg/kg bw/d for males and females.

B.6.3.2 Oral studies in mice

4-Week Toxicity Study in Mice:

Reference: *Amyes S. J. et al; 1983;* Report No. 83/ISK036/067

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is acceptable.

GLP: The study is in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060), specific GLP standards not indicated.

Material and method:

Groups of 10 male and 10 female mice (strain: CD-1; source: Charles River U.K., Margate, England) received diets containing 0, 10, 50, 250 and 3000 ppm fluazinam (batch 8203, purity 96.3 %), equivalent to 0, 1.6, 7.9, 39.5 and 455 mg/kg bw for 4 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed twice daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption was measured weekly, body weights were assessed on the first day of treatment, at twice weekly intervals thereafter and on the day of necropsy. Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, PCV, WBC total and differential), clinical chemistry (glucose, urea nitrogen, total protein, albumin, globulin, cholesterol, AP, ALT, phospholipids) and urinanalysis (appearance, volume, pH, specific grafity, glucose, bilirubin, protein, ketones, blood, urobilinogen, sediment) parameters were evaluated for all surviving animals after 3 weeks of treatment. A necropsy was done on each animal that died and on all surviving animals after 4 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, spleen, testes) were recorded. Histopathological examinations were performed on brain, heart, kidneys, marrow smear, liver, lungs with main stem bronchi, spleen and testes.

Findings:

General observations: There were no treatment-related clinical signs.

Body weight gains of males receiving 3000 ppm and 250 ppm were significantly lower than those of the respective control values during the first 10 days of treatment.

Food consumption was generally marginally lower in treated groups of both sexes.

Evaluation of hematological parameters revealed no notable or statistically significant differences from controls.

Concerning clinical chemistry, cholesterol concentrations of both sexes at dose levels of 3000 ppm were significantly higher compared to controls. Phospholipid concentrations of females receiving 3000 ppm were also significantly higher compared to controls.

Phospholipid concentrations of males from all dosing groups were higher than those for control animals but failed to attain a level of statistical significance. Glucose concentrations were higher than controls for female mice in all dosing groups except the lowest concentration (10 ppm).
(table 6.3.2.-1)

Table 6.3.2-1: Relevant clinical chemistry findings (group mean values) in mice after 4 weeks of treatment with fluazinam

Parameter	Dose group level (ppm)									
	Males					Females				
	0	10	50	250	3000	0	10	50	250	3000
Cholesterol (mg%)	110	112	120	122	135[a]	74	69	78	74	96[b]
Phospholipid (mg%)	209	219	234	242	251	152	148	151	154	200[b]
Glucose (mg%)	208	208	205	209	214	179	182	197	202[a]	205[a]

a: significantly different from controls at p<0.05; b: significantly different from controls at p<0.01 (Student's t-test)

Urinanalysis: there was no evidence of treatment-related changes at any dose group when compared to control animals.
Absolute and relative organ weight analysis after 4 weeks of treatment revealed statistically significant higher liver weights in males and females receiving 3000 ppm compared to controls. Absolute and relative kidney weight of females of the 250 ppm and 3000 ppm dosing groups was also higher than controls
(table 6.3.2-2).

Table 6.3.2-2: Body weights (g), absolute organ weights (g) and relative organ weights (expressed as a percentage) of mice after 4 weeks of treatment with fluazinam (group mean values)

	Dose group level (ppm)									
	Males					Females				
	0	10	50	250	3000	0	10	50	250	3000
Body weight	36	37	36	36	37	27	27	25[a]	27	27
Liver (abs.)	2.16	2.05	2.03	2.01	2.63[c]	1.52	1.47	1.30[a]	1.52	1.91[c]
Liver (rel.)	6.0	5.6	5.6	5.6	7.2[c]	5.6	5.6	5.1	5.7	7.0[c]
Kidney (abs.)	0.56	0.58	0.57	0.54	0.58	0.35	0.37	0.35	0.38	0.40[b]
Kidney (rel.)	1.58	1.59	1.57	1.52	1.59	1.28	1.38	1.36	1.43[b]	1.47[b]

a: significantly different from controls at p<0.05; b: significantly different from controls at p<0.01; c: significantly different from controls at p<0.001 (Student's t-test)

At necropsy, macroscopic examinations revealed no treatment-related changes. Histopathological examinations revealed a higher incidence of periacinar hepatocytic hypertrophy among treated male mice, reaching statistical significance in the highest dosing group only. A slight increase was also seen in females of the highest dosing group, statistical significance was not reached.

Table 6.3.2-3: Incidences of microscopic findings in mice after 4 weeks of treatment with fluazinam

	0		10		50		250		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Periacinar hypertrophy	1/10	0/10	3/10	0/10	6/10	0/10	6/10	0/10	10/10*	2/10

*: significantly different from controls at p<0.001 (Fisher's Exact Test)

Conclusion:

Based on clinical chemistry parameters, the NOAEL for fluazinam fed to mice for 4 weeks was 10 ppm, 1.6 mg/kg bw/d for males and females.

Toxicity to Mice by Dietary Administration for 4 Weeks:

Reference: *Chambers P. R. et al; 1994;* Report No. ISK 49/921049 and Addendum 1 – 4, ISK 49/921049, Addendum 5, *1998.*

Guideline: The study was conducted according to Japanese MAFF, 59 NohSan No. 4200 (1985), U.S. EPA/FIFRA Pesticide Assessment guidelines, Subdivision F (1984) and OECD guideline 407 (1981) and is in compliance with GLP. The study is considered acceptable..

Material and method:

Groups of 14 male and 14 female mice (strain: CD-1; source: Charles River, Michigan, USA) received diets containing 0, 3000, 5000 and 7000 ppm fluazinam (Lot 1030/91; purity 97 %), equivalent to 0, 607, 994 and 1302 mg/kg bw for 4 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed once daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption was measured weekly, body weights were assessed one week before commencement, on the first day of treatment and once a week thereafter. Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, PCV, WBC (total and differential) and clinical chemistry (bilirubin, glucose, urea nitrogen, creatinine, total protein, albumin, globulin, cholesterol, AP, ALT, AST, phospholipids, sodium, potassium, chloride and calcium) parameters were evaluated for all surviving animals after 3 weeks of treatment. A necropsy was done on each animal that died and on all surviving animals after 4 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, kidneys, liver, testes and epididymides) were recorded. Histopathological

examinations were performed on kidneys, liver, lungs and all macroscopic abnormal tissues. In addition, a microscopic reevaluation of brain tissue and cervical spinal cord from all mice was conducted (Addendum 5). Slides were reviewed by the study pathologist and then subjected to a peer review by a second pathologist:

Findings:

General observations: There were no treatment-related clinical signs.

Body weight, body weight gains and food consumption were compareable to the respective control values.

Evaluation of hematological parameters revealed no differences from control values which were considered to be related to treatment with fluazinam.

Concerning clinical chemistry, cholesterol levels of males of the 5000 and 7000 ppm groups were significantly reduced, of females significantly increased compared to controls. Male mice of the 7000 ppm dosing group showed also significantly higher glucose concentrations and reduced AST-values compared to controls. Bilirubinconcentrations were reduced in males of all dosing groups.

(table 6.3.2.-4)

Table 6.3.2-4: Relevant clinical chemistry findings (group mean values) in mice after 4 weeks of treatment with fluazinam

Parameter	Dose group level (ppm)							
	Males				Females			
	0	3000	5000	7000	0	3000	5000	7000
Cholesterol (mg%)	137	129	113	114	98	113	124*	118*
Glucose (mg%)	189	212	207	220*	187	177	179	176
Bilirubin mg/dl	0.2	0.1**	0.1**	0.1**	0.2	0.2	0.1	0.2
AST mU/ml	47	44	44	32*	45	47	52	47

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01 (Student's t-test)

Absolute and relative organ weight analysis after 4 weeks of treatment revealed statistically significant higher liver weights when adjusted for body weight in males and females of all dosing groups compared to controls. Kidney weight analysis showed also statistical significant higher values for males of the 5000 and 7000 ppm dosing groups when adjusted for body weight.

At necropsy, macroscopic examinations revealed increased incidences of pale liver and accentuated lobular markings for all treated male groups and for females receiving 7000 ppm. Liver enlargement was noted in both sexes of the 7000 ppm group.

Histopathological examinations revealed a higher incidence of centrilobular hepatocytic

hypertrophy among all treated male mice and among the majority of treated females. Kidneys of males of the 5000 and 7000 ppm groups showed minimal increased height of cortical tubular epithelium.

A statistically significant increase of vacuolation of white matter in the brain was seen in males of all dosing groups and in females of the 7000 ppm group. Males of all dosing groups showed also vacuolation of white matter in the spinal cord, statistically significant for 5000 and 7000 ppm groups. Females of the 5000 ppm group showed a slight increase of white matter vacuolation in the cerebrum, spinal cords of this dose group showed no treatment-related effect.

Table 6.3.2-5: Incidences of microscopic findings in mice after 4 weeks of treatment with fluazinam

	0 ppm		3000 ppm		5000 ppm		7000 ppm	
	♂	♀	♂	♀	♂	♀	♂	♀
Centrilobular hypertrophy	0/14	0/14	14/14	9/14	14/14	13/14	14/14	14/14
Increased height of cortical tubular epithelium	0/14	0/14	0/14	0/14	2/14	0/14	7/14	0/14
Vacuolation of white matter in the cerebrum	5/14	2/14	14/14	7/14	14/14	5/14	14/14	14/14
Vacuolation of white matter in the cerebellum	7/14	6/14	14/14	7/14	13/14	6/14	14/14	12/14
Vacuolation of white matter in the spinal cord	6/14	3/14	10/14	5/14	14/14	1/14	14/14	4/14

Conclusion:

Dietary administration of fluazinam at dose levels of 3000, 5000 and 7000 ppm for 4 weeks resulted in treatment related changes, principally in the liver, in mice of all dosing groups. On the basis of this data, it was considered that an inclusion level of 7000 ppm (1302 mg/kg bw/d) could be employed as a high dose level for a long term mouse carcinogenicity study.

B-1216: Preliminary Toxicity Study in Mice by Dietary Administration for 13 Weeks:

Reference: *Dawe S. et al; 1985;* Report No. ISK 7/85172

Guideline: The study was conducted according to Japanese MAFF, 59 NohSan No. 4200 (1985), U.S. EPA/FIFRA Pesticide Assessment guidelines, Subdivision F (1984) and OECD guideline 407 (1981) and is in compliance with GLP. Due to the lack of hematological and clinical chemistry reports, it serves only as a range finding analysis for a long term mouse carcinogenicity study.

Material and method:

Groups of 12 male and 12 female mice (strain: CD-1; source: Charles River, Kent, U.K.) received diets containing 0, 1, 10, 100 and 1000 ppm fluazinam (Lot 8303/91; purity 98.5 %), equivalent to 0, 0.13, 1.23, 14.40 and 135.28 mg/kg bw for males and 0, 0.15, 1.58, 15.07

and 152.45 mg/kg bw for females for 13 weeks. An additional group of 5 male and 5 female mice served as a health check group and were killed before treatment commenced and subjected to routine macroscopic examination. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed daily for clinical signs or reaction to treatment for the first four weeks and weekly for body weight gain and food consumption. After four weeks, clinical signs were determined on a weekly basis. A necropsy was done on each animal that died and on all surviving animals after 13 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of livers and kidneys were recorded. Histopathological examinations were restricted to livers from all mice in each group and kidneys from all mice in the control and high dose groups, and all macroscopic abnormal tissues in any animal.

Findings:

General observations: There were no treatment-related clinical signs.

Males gained more body weight than controls, females gained less weight than controls. Differences were statistically significant for males receiving 100 ppm and for females receiving 1, 10 and 1000 ppm. Neither sex showed a dose relationship. There were no statistically significant differences in food consumption.

Absolute and relative organ weight analysis after 4 weeks of treatment revealed statistically significant higher liver weights when adjusted for body weight for males of all dosing groups and for females receiving 1000 ppm compared to controls. Kidney weight analysis showed also statistical significant higher values for females of the 10, 100 and 1000 ppm dosing groups when adjusted for body weight compared to controls, but no similar effect was noted for males at any dose level.

Histopathological examinations revealed no treatment related changes.

Conclusion:

Given the results of the study, it was considered that 1000 ppm (≈150 mg/kg bw/d) could be employed as a high dose level for a long term tumorigenicity study in mice.

B.6.3.3 Oral studies in dogs

4-Week Preliminary Toxicity Study in Oral Administration to Beagle Dogs:

Reference: *Broadmeadow A. et al; 1984;* Report No. 84/ISK038/140, 85/ISK038/050 (Addendum I), 85/ISK038/248 (Addendum II)

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is acceptable as supplementary information.

GLP: The study is in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060), specific GLP standards not indicated.

Material and method:

Groups of 2 male and 2 female beagle dogs (source: Balbeggie Kennels, Fife, Scotland) received fluazinam (batch 8303-2, purity 98.5 %) in gelatine capsules daily at dosages of 1, 5, 25 and 150 mg/kg/d bw for 4 weeks. 2 male and 2 female beagle dogs served as controls, receiving empty capsules only.

Animals were observed regularly throughout the working day for clinical signs or reaction to treatment. Body weights were recorded twelve and five days before commencement, on the first day of treatment and twice weekly thereafter throughout the treatment period. Food consumption, i.e., weight of food refused, was determined daily. Before commencement of treatment and after three weeks of treatment, a physical examination was done (including an ophthalmoscopic examination, urin and blood analyses). For the ophthalmoscopic examination, pupils were dilated with 1.0 % tropicamide, then the eyes were examined with an indirect ophthalmoscope. For haematology, hematocrit, hemoglobin, PT, PTTK, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC total and differential, and for clinical chemistry, glucose, urea nitrogen, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT, AST, -GGT, OCT, CPK, creatinine concentration, phospholipids, sodium, potassium, chloride and calcium were evaluated. For urinanalysis, appearance, volume, pH, specific grafity, glucose, bilirubin, protein, ketones, blood, urobilinogen, nitrite, sediment were evaluated. At the end of the treatment period, animals were sacrificed and necropsied. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroid with parathyroid, thymus, uterus) were recorded. Histopathological examinations were performed on adrenals, aorta, brain, bronchi, caecum, colon, duodenum, epididymides, eyes and optic nerves, femur, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, cervical, peribronchial and mesenteric lymph node, mammary gland, skeletal muscle, ovaries, oesophagus, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skin, spinal cord, spleen, sternum and bone marrow, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and cervix, vagina and any tissue showing macroscopic abnormality.

A reevaluation of slides of the eyes from all dogs was conducted blind (addendum II). This examination was undertaken to investigate the incidence and severity of pigment epithelium dystrophy and basophilic inclusions.

Findings:

General observations: There were no signs of reaction to treatment and no animals died.

Food consumption and body weight gains of dogs receiving 150 mg/kg bw/d were slightly lower than those of the respective controls.

Ophthalmoscopic examinations revealed grey pigmentation, slight or scattered, of the tapetal fundus in all dogs receiving 150 mg/kg bw/d, in one male receiving 25 mg/kg bw/d and in one male receiving 1 mg/kg bw/d.

Evaluation of hematological and clinical chemistry parameters and urinanalysis did not

reveal any changes attributable to treatment.

Organ weight analysis after 4 weeks of treatment revealed high bodyweight-relative liver weights in males and females receiving 150 mg/kg bw/d and in one male and both females receiving 25 mg/kg bw compared to controls.

At necropsy, macroscopic and histopathological examinations revealed no changes which could be attributed to treatment with fluazinam. A slight to moderate dystrophy of the pigment epithelium of the retina, with or without basophilic inclusions, was present in the majority of dogs, including controls.

Table 6.3.3-1: Incidences of microscopic findings in the eyes of dogs after 4 weeks of treatment with fluazinam

Mg/kg/d	0		1		5		25		150	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
pigment epithelium dystrophy	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
basophilic inclusions	1/2	2/2	0/2	1/2	1/2	1/2	2/2	1/2	0/2	1/2

Conclusion:

There was evidence of toxic change in dogs receiving 150 mg/kg bw/d and to a lesser extent in those receiving 25 mg/kg bw/d (reduced food intake and bodyweight in animals receiving 150 mg/kg bw/d and increased liver weights in animals receiving 25 and 150 mg/kg bw/d). All dogs receiving 150 mg/kg bw/d showed also grey pigmentation of the tapetal fundus of the retina. In this study, the NOAEL for fluazinam was considered to be 5 mg/kg bw/d.

13 -Week Toxicity Study in Oral Administration to Beagle Dogs:

Reference: *Broadmeadow A. et al; 1985;* Report No. 84/ISK048/692; Amended final report No. 91/ISK048/0832 and Addendum 3 (1998)

Guideline: The study was conducted according to U.S. EPA Guideline 82-1 and is in compliance with GLP. The study is considered acceptable.

Material and method:

Groups of 4 male and 4 female beagle dogs (source: Balbeggie Kennels, Fife, Scotland) received fluazinam (batch 8303-2, purity 98.5 %) in gelatine capsules daily at dosages of 1, 10 and 100 mg/kg/d bw for 13 weeks. 4 male and 4 female beagle dogs served as controls, receiving empty capsules only.

Animals were observed regularly throughout the working day for clinical signs or reaction to treatment. Body weights were recorded at weekly intervals throughout the acclimatisation and treatment periods. Food consumption, i.e., weight of food refused, was determined daily. Before commencement of treatment and after 4, 8 and 12 weeks of treatment, a physical examination was done. An ophthalmoscopic examination was performed before treatment

and after 6, 9 and 12 weeks of treatment. For the ophthalmoscopic examination, pupils were dilated with 1.0 % tropicamide, then the eyes were examined with an indirect ophthalmoscope. Urin and blood analyses were done before commencement and after 6 and 12 weeks of treatment. For haematology, hematocrit, hemoglobin, PT, PTTK, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC total and differential, and for clinical chemistry, glucose, urea nitrogen, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT, AST, -GGT, OCT, CPK, creatinine concentration, phospholipids, sodium, potassium, chloride and calcium were evaluated. For urinanalysis, appearance, volume, pH, specific grafity, glucose, bilirubin, protein, ketones, blood, urobilinogen, nitrite, sediment were evaluated. At the end of the treatment period, animals were sacrificed and necropsied. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroid with parathyroid, thymus, uterus) were recorded. Histopathological examinations were performed on adrenals, aorta, brain, bronchi, caecum, colon, duodenum, epididymides, eyes and optic nerves, femur, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, cervical, peribronchial and mesenteric lymph node, mammary gland, skeletal muscle, ovaries, oesophagus, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skin, spinal cord, spleen, sternum and bone marrow, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and cervix, vagina and any tissue showing macroscopic abnormality. In addition, a microscopic reevaluation of brain tissue and cervical spinal cord from all control and high dose level dogs of this study was conducted (Addendum 3, 1998). Slides were reviewed by the study pathologist and then subjected to a peer review by a second pathologist:

Findings:

General observations: There were no signs of reaction to treatment and no animals died during the treatment period.

Food consumption was lower in one female of the high dose group, body weight gains of two males and one female of the high dose group (100 mg/kg bw/d) were slightly lower than those of the respective controls. Among dogs receiving 10 mg/kg bw/d, one male and one female had marginally lower weight gains.

Ophthalmoscopic examinations after 6 weeks of treatment revealed, in the retina, grey mottling of the tapetal fundus in 3 male and 3 female dogs receiving 100 mg/kg bw/d, associated with slight retinal hyperreflection. After 9 and 12 weeks of treatment grey mottling of the tapetal fundus was detected in all dogs of this dose group, although association with retinal hyperreflection was less clear.

Table 6.3.3-2: Ophthalmoscopic findings in the eyes of dogs after 6, 9 and 12 weeks of treatment with fluazinam

Parameter	Dose group level (mg/kg bw/d)									
	Males					Females				
	Week	0	1	10	100	Week	0	1	10	100
Grey mottling of tapetal fundus	0	0/4	0/4	0/4	0/4	0	0/4	0/4	0/4	0/4
	7	1/4	0/4	0/4	3/4	7	0/4	1/4	0/4	3/4
	10	0/4	0/4	0/4	4/4	10	0/4	0/4	0/4	4/4
	13	0/4	0/4	0/4	4/4	13	0/4	0/4	1/4	4/4
Retinal hyperreflection	0	0/4	0/4	0/4	0/4	0	0/4	0/4	0/4	0/4
	7	0/4	0/4	0/4	3/4	7	0/4	0/4	0/4	3/4
	10	0/4	0/4	1/4	3/4	10	0/4	0/4	1/4	0/4
	13	0/4	0/4	0/4	2/4	13	0/4	0/4	0/4	1/4

Evaluation of hematological and urinanalysis did not reveal any changes attributable to treatment. Clinical chemistry parameters revealed high plasma alkaline phosphatase activity (AP) in animals of the high dose group.

Organ weight analysis after 13 weeks of treatment revealed high absolute and bodyweight-relative liver weights in animals receiving 100 mg/kg bw/d compared to controls.

At necropsy, macroscopic examinations revealed no changes which could be attributed to treatment with fluazinam.

Histopathological changes were confined to the livers of dogs of the high dose group, expressed as bile duct hyperplasia with and without cholangiofibrosis in 2 males and 2 females.

A slight to moderate dystrophy of the pigment epithelium of the retina was present in the majority of dogs, including controls. There was no effect of treatment on the incidence or degree of vacuolation of white matter in the brain or spinal cord of dogs of the high dose group (100 ppm) compared to controls.

Table 6.3.3-3: Histopathological findings in dogs after 13 weeks of treatment with fluazinam

Mg/kg bw/d	0		1		10		100	
	♂	♀	♂	♀	♂	♀	♂	♀
Bile duct hyperplasia	0/4	0/4	0/4	1/4	0/4	0/4	2/4	2/4
pigment epithelium dystrophy	4/4	4/4	4/4	4/4	4/4	4/4	4/4	3/4

Conclusion:

Oral administration of 100 mg/kg bw/d fluazinam to beagle dogs for 13 weeks caused changes in absolute and relative liverweight and histopathological changes in this organ. Ophthalmoscopic examinations revealed changes in the retina at the highest dose level.

Histopathologically, dystrophy of the pigment epithelium of the retina was present in the majority of dogs, including controls. Based on these results, the NOAEL for this study was considered to be 10 mg/kg bw/d.

11-Week Oral Toxicity Study in Dogs to Investigate possible Changes in Retinal Function and Morphology and the Reversibility of such Changes:

Reference: *Hull R. M. et al; 1986;* Report No.CTL/C/1778

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is acceptable as supplementary information and is in compliance with GLP.

Material and method:

A group of six male beagle dogs (source: Alderley Park) received fluazinam (batch 8303-2, purity 98.5 %) in gelatine capsules at a dose level of 200 mg/kg/bw/d for 11 weeks. 6 male beagle dogs served as controls, receiving empty capsules only. Due to overt toxicity, dose levels were reduced to 150 mg/kg bw/d in weeks 3 to 5. After 11 weeks administration, 3 dogs per group were terminated (main group) and the remaining 3 dogs per group (withdrawal group) continued without treatment for 5 weeks for further investigation.
Animals were observed twice daily for clinical signs or reaction to treatment. Body weights were recorded at weekly intervals throughout the acclimatisation and treatment periods. Food consumption, i.e., weight of food refused, was determined daily. Before commencement and during week 4 and 10 of treatment, a physical examination was done. An ophthalmoscopic examination was performed before treatment and on day 8, 14, 21, 28, 44, 50, 58, 64 and 71 of treatment. For the ophthalmoscopic examination, pupils were dilated with 0.5 % tropicamide, then the eyes were examined with an indirect ophthalmoscope. Electroretinographies (ERG) were recorded from all animals during week 10 and from all compound withdrawal animals during week 15.
Blood analyses were done before commencement and on day 8, 15, 22, 43, 57, 65 and 75 of treatment and also on day 78, 86, 92, 99 and 105 for all compound withdrawal animals. For haematology, hematocrit, hemoglobin, PT, PTTK, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC total and differential, and for clinical chemistry, urea, total protein, albumin, total bilirubin, cholesterol, triglycerides, AP, ALT, AST and creatinine concentration were evaluated. For urinanalysis, appearance, volume, pH, specific grafity, glucose, bilirubin, protein, ketones, blood, urobilinogen, nitrite, sediment were evaluated. At the end of the treatment period, animals were sacrificed and only the eyes examined histopathologically (right eye) and via electron microscopy (left eye).

Findings:

During the dosing period, food intake and body weight gains were reduced, but were reversed during the compound withdrawal period.
Evaluation of hematological and urinanalysis did not reveal any changes attributable to treatment. Clinical chemistry parameters revealed increases in ALT and ALP levels, indicating hepatotoxicity. Levels were reduced to near pre-study values during the

withdrawal period.

Ophthalmoscopic examinations revealed increased brown granularity of the tapetal fundus of both eyes in one animal of the main group on day 14 and was present until termination on day 71. One dog of the withdrawal group showed brown granularity of the tapetal fundus of both eyes from day 21 onwards. In the fourth week of compound withdrawal (day 104) brown granularity was less apparent.

ERG findings showed a decrease in the amplitude of the ERG without alteration in other ERG parameters. The reduction in ERG voltage was almost completely reversed in 2 of 3 dogs by the end of the withdrawal period but not in the remaining dog. There was no evidence of nyctalopia or modification of the ERG waveform which would imply neural damage. No compound related changes were seen at histological or electron microscope examination of the retina.

Conclusion:

Oral administration of 200/150 mg/kg bw/d fluazinam to beagle dogs for 11 weeks caused changes in clinical chemical parameters, indicating hepatotoxicity. Ophthalmoscopic examinations revealed ERG-abnormalities which can be accounted for by functional changes in the pigment epithelium of the retina. The results show recovery of response amplitude after withdrawal of fluazinam, but it is not possible to say if recovery would be complete.

52 -Week Toxicity Study in Oral Administration to Beagle Dogs:

Reference: *Broadmeadow A. et al; 1987;* Report No. 86/ISK055/512, Addendum 1 (1998)

Guideline: No specific test guideline is mentioned in the study. Nevertheless, the study is in compliance with GLP and considered acceptable.

Material and method:

Groups of 6 male and 6 female beagle dogs (source: Animal Breeding Unit, Imperial Chemical Industries plc, Pharmaceuticals Division, Alderley Park, Cheshire, England) received fluazinam (batch 8412-20, purity 95.3 %) in gelatine capsules daily at dosages of 1, 10 and 50 mg/kg/d bw for 52 weeks. 6 male and 6 female beagle dogs served as controls, receiving empty capsules only.

Animals were observed regularly throughout the working day for clinical signs or reaction to treatment. Body weights were recorded at weekly intervals throughout the acclimatisation and treatment periods. Food consumption, i.e., weight of food refused, was determined daily. Before commencement of treatment and after 12, 24, 38 and 50 weeks of treatment, a physical examination was done. An ophthalmoscopic examination was performed before treatment and after 12, 24 and 50 weeks of treatment. For the ophthalmoscopic examination, pupils were dilated with 1.0 % tropicamide, then the eyes were examined with an indirect ophthalmoscope. At the same frequency as for ophthalmoscopy, photographs were taken of the retina, including the tapetum, of both eyes of each animal. Urin and blood analyses were done before commencement and after 12, 23 and 50 weeks of treatment. For haematology,

hematocrit, hemoglobin, PT, PTTK, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC total and differential, and for clinical chemistry, glucose, urea nitrogen, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT, AST, -GGT, OCT, CPK, creatinine concentration, phospholipids, sodium, potassium, chloride and calcium were evaluated. For urinanalysis, appearance, volume, pH, specific grafity, glucose, bilirubin, protein, ketones, blood, urobilinogen, nitrite, sediment were evaluated. At the end of the treatment period, animals were sacrificed and necropsied. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, spleen, testes, thyroid with parathyroid, thymus, uterus) were recorded. Histopathological examinations were performed on adrenals, aorta, brain, bronchi, caecum, colon, duodenum, epididymides, eyes and optic nerves, femur, gall bladder, heart, ileum, jejunum, kidneys, liver, lungs, cervical, peribronchial and mesenteric lymph node, mammary gland, skeletal muscle, ovaries, oesophagus, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skin, spinal cord, spleen, sternum and bone marrow, stomach, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus and cervix and any tissue showing macroscopic abnormality. In addition, a microscopic reevaluation of brain tissue and cervical spinal cord from all control and treated dogs of this study was conducted (Addendum 1, 1998). Slides were reviewed by the study pathologist and then subjected to a peer review by a second pathologist:

Findings:

General observations: No animals died. Signs of reaction to treatment were salivation in both sexes of the high dose group and nasal dryness in females of the high and mid dose groups. Food consumption was lower in one female of the high dose group, body weight gains of males and females of the high dose group were slightly lower than those of the respective controls. Ophthalmoscopic examinations revealed no treatment-related ocular lesions.
Hematology: packed cell volume, hemoglobin concentrations and erythrocyte counts of high dose dogs were significantly lower throughout the treatment period. After 23 weeks of treatment, also females receiving 10 mg/kg bw/d were affected, but these effects were not apparent after 50 weeks of treatment. After 12 weeks of treatment, the total leucocyte counts of males receiving 50 mg/kg bw/d were statistically significantly higher compared to controls. After 23 weeks of treatment, this change was extended to males receiving 10 mg/kg bw/d and to females of the high dose group. After 50 weeks of treatment, both sexes of the mid and high dose groups were affected.
Examination of bone marrow smears indicated a statistically significant increase in myeloid to erythroid ratio in females of the mid and high dose groups.
Clinical chemistry parameters revealed high plasma alkaline phosphatase activity (AP) in animals of the high dose group and increases of cholesterol levels. Plasma albumin concentrations were lower in both sexes of the high dose group compared to controls. Males

of the high dose group showed also slightly lower glucose concentrations at weeks 13 and 24 (see table 6.3.3.-4)

Table 6.3.3-4: Relevant haematological and clinical chemistry findings (group mean values) in dogs after treatment with fluazinam

Parameter	Dose group level (mg/kg bw/d)									
	Males					Females				
	Week	0	1	10	50	Week	0	1	10	50
Packed cell volume %	0	44	41	44	43	0	41	42	42	42
	12	43	43	43	39**	12	44	45	43	40*
	23	47	46	46	43*	23	50	48	46*	43***
	50	46	44	43	41*	50	44	46	43	40*
Hemoglobin G%	0	14.1	13.6	14.3	14.0	0	13.5	13.7	13.6	13.3
	12	14.1	14.1	14.2	13.1*	12	14.4	14.7	14.1	13.2*
	23	15.9	15.5	15.7	14.6*	23	16.6	16.0	15.6	14.4**
	50	16.0	15.5	15.5	14.6*	50	15.4	16.1	14.9	14.3
RBC ml/mm^3	0	6.41	6.09	6.47	6.21	0	6.10	6.28	6.16	6.07
	12	5.92	5.89	5.94	5.37**	12	6.15	6.22	5.87	5.47**
	23	6.92	6.64	6.73	6.13*	23	7.33	7.10	6.67*	6.10***
	50	6.80	6.50	6.37	5.99*	50	6.58	6.79	6.19	5.89*
WBC 1000/ mm^3	0	13.4	14.7	14.1	14.6	0	14.0	13.6	14.1	13.4
	12	11.8	11.2	14.3	15.2*	12	12.1	12.7	13.1	14.4
	23	12.4	15.2	15.9*	17.0**	23	13.3	14.2	15.9	17.3*
	50	11.6	12.2	15.4*	19.0***	50	13.0	15.1	17.1*	18.3*
Cholesterol mg%	0	178	153	154	182	0	161	149	157	171
	12	159	162	154	199	12	142	156	147	164
	23	172	167	163	201	23	157	167	167	192
	50	159	150	138	198*	50	169	152	187	175
Glucose mg%	0	110	108	110	108	0	110	109	113	113
	12	104	104	106	96**	12	100	105	104	99
	23	111	107	106	99***	23	111	105*	105*	106*
	50	104	100	104	99	50	106	106	105	104
Albumin g%	0	3.1	3.1	3.0	3.1	0	3.1	3.0	3.1	3.1
	12	3.3	3.2	2.9	3.0	12	3.1	3.0	3.2	3.1
	23	3.1	3.0	3.1	2.7**	23	3.4	3.3	3.2	3.0*
	50	3.2	3.4	3.1	2.9	50	3.4	3.4	3.2	3.1*
Alkaline phosphatase	0	84	94	96	103	0	90	94	93	90
	12	64	70	82	121***	12	64	66	68	97*

Parameter	Dose group level (mg/kg bw/d)									
	Males					Females				
	Week	0	1	10	50	Week	0	1	10	50
iu/l	23	52	60	73	147***	23	57	52	61	107**
	50	39	40	53	109***	50	46	39	51	75*

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01; ***: significantly different from controls at p<0.001 (Student's t-test)

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Urinanalysis did not reveal any changes attributable to treatment.

Organ weight analysis after 52 weeks of treatment revealed high absolute and bodyweight-relative liver weights in all animals receiving 50 mg/kg bw/d and in 2 females receiving 10 mg/kg bw/d compared to controls.

At necropsy, macroscopic examinations revealed an increased incidence and extent of liquefied contents of the gastro-intestinal tract in both sexes of the mid and high dose groups compared to controls. Numerous pale masses were observed in the pyloric mucosa of one high dose male and was considered to be associated with lymphoid hyperplasia noted microscopically. The gall bladder of one male and three females of the high dose group contained dark, free masses, partly a distension of the gall bladder was observed.

Histopathological changes were observed in the stomach of males of the high and mid dose groups, expressed as lymphoid hyperplasia of the mucosa.

No histopathological lesion was observed in the retina.

Male and female dogs of the high dose group showed an increased incidence or degree of vacuolation of white matter in the cerebrum, cerebellum, pons, medulla and midbrain. Females of the high dose group showed also vacuolation of white matter in the spinal cord. No vacuolation of white matter was observed in the spinal cord of males. Animals of both sexes of the mid and low dose groups had no effects of treatment in the brain or spinal cord compared to controls.

Table 6.3.3-5: Incidence and Degree of White Matter Vacuolation in the Central Nervous System of the Dog

		Male				Female			
Dosage level (mg/kg/day)		0	1	10	50	0	1	10	50
Cerebrum									
No abnormalities detected		3	3	4	0	4	4	3	0*
Vacuolation of white matter	Total	3	3	2	6	2	2	3	6*
	Trace	3	3	2	1	2	2	3	1
	Minimal	0	0	0	5**	0	0	0	3
	Moderate	0	0	0	0	0	0	0	2

Cerebellum/pons/medulla/midbrain									
No abnormalities detected		3	3	4	0	2	2	0	0
Vacuolation of white matter	Total	3	3	2	6	4	4	6	6
	Trace	3	3	2	6	4	4	6	3
	Minimal	0	0	0	0	0	0	0	3
Spinal cord									
No abnormalities detected		6	6	6	6	6	6	6	2
Vacuolation of white matter	Total	0	0	0	0	0	0	0	4*
	Trace	0	0	0	0	0	0	0	3
	Minimal	0	0	0	0	0	0	0	1
Number of dogs examined		6	6	6	6	6	6	6	6

*Statistically significant difference from control, p<0.05, ** p<0.01 (Fisher's Exact Test)

Conclusion:

Oral administration of 50 mg/kg bw/d fluazinam to beagle dogs for 52 weeks produced an increase of liver weight and accompanying blood chemistry changes. Also changes in some hematological parameters were observed. An increased incidence of liquefied contents of the gastro-intestinal tract and mucosal lymphoid hyperplasia of the stomach were also noted. These changes were also observed partly in animals of the mid dose group.

Animals of the high dose group showed vacuolation of white matter in the brain. Females of the high dose group showed also vacuolation of white matter in the spinal cord. Animals of both sexes of the mid and low dose groups had no effects of treatment in the brain or spinal cord compared to controls. 1 mg/kg bw/d was considered to be the NOAEL in this study.

B.6.3.4 Dermal study in rats

B-1216: 21-Day Percutaneous Toxicity Study in CD Rats

Reference: *Cummins, H.A.; 1985;* Report No. 84/ISK052/690; Amended final report No. 91/ISK052/0824

Guideline: The study was performed in accordance with U.S. EPA Guideline 82-2 and is in compliance with GLP. The study is considered acceptable.

Material and Methods:

Groups of 10 rats/sex (strain: Sprague-Dawley (CD); source: Charles River (U.K.) Limited) received doses of 10, 100 and 1000 mg/kg bw fluazinam (batch no. 8303-2; purity 98.5 %) by occluded application to the shaven skin for 6 hours per day for 21 days. An additional group of 10 males and 10 females received the vehicle only, 0.5% methyl cellulose, to serve as controls. Animals were observed for clinical signs and mortality 4 times per day and dermal reactions were assessed daily. Body weight and food consumption were recorded

weekly. Hematology and blood chemistry were analyzed on day 20. All animals were necropsied and the weights of selected organs (adrenals, brain, kidneys, liver, ovaries, testes) were recorded. Histopathological examinations were performed on heart, kidneys, liver, lungs, ovaries, skin, stomach and testes and any tissue showing macroscopic abnormality.

Findings:

General observations: there were no external systemic signs of reaction to treatment.

Food consumption: no differences in food consumption were observed between treated and control animals, body weight gains of males of the high dose group were slightly lower than those of the respective controls.

Hematology: no differences were observed between treated and control animals.

Clinical chemistry parameters revealed statistically significant higher aspartate amino transferase activity (AST) in both sexes of the high dose group and in males of the intermediate and low dose groups. Cholesterol levels of both sexes of the high dose group and of males of the intermediate group were also statistically significantly higher compared to controls.

Organ weight analysis after 3 weeks of treatment revealed higher absolute and relative liver weights in all animals receiving 1000 mg/kg bw/d compared to controls.

At necropsy, macroscopic examinations revealed encrustations or staining of the skin at the treatment site in both sexes of the high and some females of the mid dose groups compared to controls.

Histopathological changes were confined to the liver and skin at the treatment site. In the liver, periacinar hepatocytic hypertrophy was present in males and females of the high dose groups and in one male of the mid dose group. Changes in treated skin comprised acanthosis, dermatitis, scabs and ulceration. Acanthosis and dermatitis were observed in animals of all dose groups, scabs and ulceration were restricted to animals of the high dose groups and to one female of the mid dose group.

Conclusion:

Repeated dermal administration of fluazinam at concentrations of 10, 100 and 1000 mg/kg bw to rats for 3 weeks revealed changes in clinical chemistry parameters, especially in males, at all dose groups. A toxic effect was also observed in the liver in both sexes of the high dose and in males of the mid dose groups. Effects to the skin (acanthosis and dermatitis) were also observed at all dose groups compared to controls, so it is not possible to consider a NOAEL for this study.

B.6.3.5 Summary of short-term toxicity studies

Subacute and subchronic administration of fluazinam to rats, mice and dogs caused reduced food consumption and body weight gain. Changes of hematological parameters such as lower haemoglobin concentrations, lower erythrocyte counts and lower platelet counts were also observed. Clinical chemistry parameters showed low ALT activity, higher cholesterol, phospholipid and glucose concentrations. Higher absolute and relative liver weights and

histopathological changes in the liver such as periacinar hepatocytic hypertrophy were observed in all species. In mice and dogs, vacuolation of white matter in brain and spinal cord was observed. High dosed dogs of the 4- and 13-week oral toxicity studies (150 and 100 mg/kg bw/d resp.) showed retinal hyperreflection and grey pigmentation of the tapetal fundus of the retina. At histopathologic examination, a dystrophy of the pigment epithelium of the retina was observed in the majority of dogs, including controls. The toxicological significance of the ophthalmic observations and the possible interrelationships between these and the retinal findings observed histopathologically were unknown. Oral administration of 200/150 mg/kg bw/d fluazinam to beagle dogs for 11 weeks revealed ERG-abnormalities which can be accounted for by functional changes in the pigment epithelium of the retina. The results show recovery of response amplitude after withdrawal of fluazinam, but it is not possible to say if recovery would be complete.

Dermal administration of fluazinam to rats for 3 weeks revealed changes in clinical chemistry parameters such as higher AST activity and higher cholesterol levels in all dose groups (10, 100 and 1000 mg/kg bw). A toxic effect was also observed histopathologically in the liver in both sexes of the high dose and in males of the mid dose groups (periacinar hepatocytic hypertrophy). Dermatitis and acanthosis of the skin were seen in all dose groups compared to controls.

Table 6.3.5-1: Summarised results of subacute/subchronic toxicity studies with fluazinam

Study; Reference	Dose levels	NOAEL	Relevant effects
CD rats 4 weeks oral *Broadmeadow A. et al; 1983*	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.26, 5.21, 26.1 and 305.4 mg/kg bw)	1.26 mg/kg bw/d	-reduced food consumption and body weight gain -hematological and clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the liver
CD rats 13 weeks oral *Broadmeadow A. et al; 1985*	0 and 500 ppm/diet (equivalent to 0 and ≈ 40 mg/kg bw/d)	Cannot be determined	-reduced body weight gain -higher relative liver weights -histopathological changes in the liver
CD rats 13 weeks oral *Broadmeadow A. et al; 1984*	0, 2, 10, 50 and 500 ppm/diet (equivalent to 0, 0.16, 0.82, 4.1 and 41 mg/kg bw)	4.1 mg/kg bw/d	-hematological findings -higher relative liver weights -higher absolute and relative lung and uterus weights -histopathological changes in the liver
CD rats 21 days dermal *Cummins H. A. et al; 1985*	0, 10,100 and 1000 mg/kg bw)	Cannot be determined	-reduced body weight gain -clinical chemical findings -higher absolute and relative liver weights -encrustations or staining of the skin -histopathological changes in the liver and skin

Study; Reference	Dose levels	NOAEL	Relevant effects
CD-1 mice 4 weeks oral *Amyes S. J. et al; 1983*	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.6, 7.9, 39.5 and 455 mg/kg bw)	1.6 mg/kg bw/d	-reduced food consumption and body weight gain -clinical chemical findings -higher absolute and relative liver and kidney weights -histopathological changes in the liver
CD-1 mice 4 weeks oral *Chambers P. R. et al; 1994*	0, 3000, 5000 and 7000 ppm/diet (equivalent to 0, 607, 994 and 1302 mg/kg bw)	Cannot be determined	-clinical chemical findings -higher absolute and relative liver and kidney weights -histopathological changes in liver and kidneys -vacuolation of white matter in brain and spinal cord
CD-1 mice 13 weeks oral *Dawe S. et al; 1985*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.13, 1.23, 14.4 and 135.28 mg/kg bw for males and 0, 0.15, 1.58, 15.07 and 152.45 mg/kg bw for females)	Cannot be determined	-higher absolute and relative liver and kidney weights
Beagle dogs 4 weeks oral *Broadmeadow A. et al; 1984*	0, 1, 5, 25 and 150 mg/kg bw, gelatine capsules)	5 mg/kg bw/d	-reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -higher relative liver weights
Beagle dogs 13 weeks oral *Broadmeadow A. et al; 1985*	0, 1,10 and 100 mg/kg bw, gelatine capsules)	10mg/kg bw/d	-reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the liver
Beagle dogs 11 weeks oral *Hull R. M. et al; 1986*	0 and 200/150 mg/kg bw, gelatine capsules)	not determined	-reduced food consumption and body weight gain -clinical chemical findings -brown granularity of the tapetal fundus of the retina -ERG-abnormalities
Beagle dogs 52 weeks oral *Broadmeadow A. et al; 1987*	0, 1, 10 and 50 mg/kg bw, gelatine capsules)	1mg/kg bw/d	-reduced food consumption and body weight gain -hematological and clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the stomach -vacuolation of white matter in brain and spinal cord

B.6.4 Genotoxicity (Annex IIA 5.4)

B.6.4.1 In vitro assays

IKF-1216 Bacterial mutation assay

Reference: *Kitching J.; 2000;* Report No.RIA 015/003043;

Guideline: The study was conducted according to OECD Guideline 471 (1997); EEC Annex to Directive 92/69/EEC (1992) Part B; U.S. EPA Health Effects Guidelines, OPPTS 870.5100, EPA 712-C-98-247; Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

Fluazinam technical (batch A629/1995, purity 98.4%) was tested in the Ames test. Histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA1535, TA1537, TA98 and TA100) and a tryptophan dependent mutant of *Escherichia coli*, strain WP2*uvr*A/pKM101 (CM891) were exposed to fluazinam diluted in dimethylsulfoxide (DMSO), which was also used as a negative control. Positive controls were, in the absence of S-9 mix, *sodium azide* (0.5 µg/plate for strains TA1535 and TA100), *9-aminoacridine* (30 µg/plate for strain TA1537), *2-nitrofluorene* (1µg/plate for strain TA98) and *2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)* (0.05 µg/plate for strain WP2uvrA/pKM101 (CM891)). In the presence of S-9 mix, *2-aminoanthracene* (2µg/plate for strain 1535) and *benzopyrene* (5 µg/plate for strains TA1537, TA98 and TA100) were used.

Three independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S-9 mix). The first and second were standard plate incorporation assays, the third involved a pre-incubation stage.

First test: The test substance was added to cultures of the five tester strains at seven concentrations separated by *ca* half-$\log_{10}$ intervals. Dose concentrations were 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The highest concentration is the standard limit dose recommended in the regulatory guidelines this assay follows. An aliquot of 0.1 ml of a bacterial culture 10-hours after cultivation and 0.5 ml S-9 mix or 0.5 ml 0.1 M sodium phosphate buffer (pH 7.4) were placed in glass bottles. An aliquot of 100 µl of the test solution was added, followed immediately by 2 ml of molten agar containing 0.05mM histidine/biotin/tryptophan. The mixture was shaken and overlaid onto petridishes containing 25 ml minimal agar. Three petridishes were used for each dose level. Plates were also prepared without the addition of bacteria in order to assess the sterility of the test substance, S-9 mix and phosphate buffer. All plates were incubated at 37° C for 72 hours. After this period the appearance of the background bacterial lawn was examined and revertant colonies counted using a Seescan automated colony counter.

Second test: As insufficient non-toxic dose levels were obtained in the first test, it was repeated using the same conditions but with a highest concentration of 50 µg/plate and a total of eight dose levels (50, 15, 5, 1.5, 0.5, 0.15, 0.050 and 0.015 µg/plate).

Third test: As a clear negative response was obtained in the second test, a variation to the test procedure was used for the third. The variation used was the pre-incubation assay in which the bottles which contained mixtures of bacteria, buffer or S-9 mix and test solution, were incubated at 37° C for 30 minutes with shaking before the addition of the agar overlay. 50 µg/plate was again chosen as the top concentration, with a total of nine dose levels (50, 15, 5, 1.5, 0.5, 0.15, 0.050, 0.015 and 0.005 µg/plate).

Evaluation criteria: For a test to be considered valid, the mean of the solvent control revertant colony numbers for each strain should lie within the 99% confidence limits of the current historical control range of the laboratory. The positive control compounds must cause at least a doubling of mean revertant colony numbers over the negative control. The mean number of revertant colonies for all treatment groups was compared with those obtained for the solvent control groups. The mutagenic activity of a test substance was assessed by applying the following criteria:

(a) If treatment with a test substance produced an increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S-9 mix, it was considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(b) If treatment with a test substance did not produce reproducible increases of at least 1.5 times the concurrent solvent controls, in either mutation test, it was considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.

(c) If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may have been performed in order to resolve the issue of the test substance's mutagenic activity in this test system. Should an increase in revertant colony numbers then be observed which satisfies paragraph (a), the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

If no clear "positive" response was obtained, the test data may have been subjected to analysis to determine the statistical significance of any observed increases in revertant colony numbers.

Findings:

In the first test, toxicity (visible thinning of the background lawn of non-revertant cells) was observed towards all tester strains at 50 µg/plate and above and towards all the *S. typhimurium* strains at 15 and 5 µg/plate in the absence of S-9 mix. In the second mutation test, toxicity was observed towards all tester strains at the highest dose level tested, 50 µg/plate, and towards all the *S. typhimurium* strains at 15 and 5 µg/plate in the absence of S-9 mix. Toxicity also was observed towards TA100 at 1.5 and 0.5 µg/plate in the absence of S-9 mix. In the third mutation test, toxicity was observed towards all tester strains at 50 and 15µg/plate and towards all the *S. typhimurium* strains at 0.5 µg/plate in the absence of S-9 mix. Toxicity also was observed towards TA100 in the presence of S-9 mix at 0.5µg/plate. No

precipitation was observed in any test.

No evidence of mutagenic activity was seen at any dose level of fluazinam in any mutation test. The concurrent positive controls demonstrated the sensitivity of the assay and the metabolizing activity of the liver preparations, inducing substantial increases in revertant colony numbers with all strains.

Conclusion:

Fluazinam showed no evidence of mutagenic activity in this bacterial system, either in the presence or absence of metabolic activation.

Bacterial reverse mutation test of fluazinam technical

Reference: *Ohtsuka M.; 1988;* Report No.T-1674E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of technical fluazinam (Lot No. 8412-20, purity 95.3%) in bacterial systems. The *S. typhimurium* (TA100, TA1535, TA98 and TA1537) test was performed with 0.0625, 0.125, 0.25, 0.5, 1 and 2 µg/plate in a DMSO solution without metabolic (S-9) activation and with 3.13, 6.25, 12.5, 25, 50 and 100 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). The *E. coli* (WP2 uvr A) test without S-9 mix was performed with 15.6, 31.3, 62.5, 125 and 250 µg/plate, and 31.3, 62.5, 125, 250 and 500 µg/plate with S-9 mix. Dose levels were established on the basis of preliminary range finding tests: Without metabolic (S-9) activation, fluazinam at dose levels of 1 and 3 µg /plate and above caused growth inhibition in *S. typhimurium strains* TA100 and TA98 respectively and with metabolic (S-9) activation at dose levels of 90 µg /plate and above. In *E. coli,* growth was inhibited at a dose of 250 µg /plate without metabolic (S-9) activation and at a dose of 500 µg /plate with metabolic (S-9) activation.

Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.

Evaluation criteria:

The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose response.

Findings:

The results of the tests showed that the number of revertant colonies for the tester strains exposed to fluazinam, at all dose levels, either with or without metabolic activation, were less

than twice that for the solvent control. AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).

Conclusion:

The results of this test indicate that fluazinam was not mutagenic in the bacterial reverse-mutation assays at the concentrations tested.

Bacterial reverse mutation test of fluazinam technical

Reference: *Ohtsuka M.; 1989;* Report No.T-1673E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of technical fluazinam (Lot No.109, purity 95.3%) in bacterial systems. The *S. typhimurium* (TA100, TA1535, TA98 and TA1537) test was performed with 0.0313, 0.0625, 0.125, 0.25, 0.5 and 1µg/plate in a DMSO solution without metabolic (S-9) activation and with 3.13, 6.25, 12.5 25, 50 and 100 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). The *E. coli* (WP2 uvr A) test without S-9 mix was performed with 15.6, 31.3, 62.5, 125 and 250 µg/plate, and with 31.3, 62.5, 125, 250 and 500 µg/plate with S-9 mix. Dose levels were established on the basis of preliminary range finding tests: Without metabolic (S-9) activation, fluazinam at a dose level of 1 µg /plate and above caused growth inhibition in *S. typhimurium strains* TA100, TA98 and TA 1535 and with metabolic (S-9) activation at a dose level of 100 µg /plate and above. In *E. coli,* growth was inhibited at a dose of 250 µg /plate without metabolic (S-9) activation and at a dose of 500 µg /plate with metabolic (S-9) activation.

Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.

Evaluation criteria:

The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose-response.

Findings:

The results of the tests showed that the number of revertant colonies for the tester strains exposed to fluazinam, at all dose levels, either with or without metabolic activation, were less than twice that for the solvent control. AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains

in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).

Conclusion:

The results of this test indicate that fluazinam was not mutagenic in the bacterial reverse mutation assays at the concentrations tested.

IKF-1216 Mammalian cell mutation assay

Reference: *Ransome S.; 2000;* Report No.RIA 017/004090;

Guideline: The study was conducted according to OECD Guideline 476 (1997); Commission Directive 2000/32/EC (2000) Annex 4E – B17 (L136, 65); U.S. EPA (1998) Health Effects Guidelines, OPPTS 870.5300, EPA 712-C-98-221and is in compliance with GLP.

The study is considered acceptable.

Material and method:

Fluazinam technical (batch A629/1995, purity 98.4%) was tested in the mouse lymphoma L5178Y cell mutation test and was diluted in dimethylsulfoxide (DMSO), which was also used as a negative control. Positive controls were, in the absence of S-9 mix, *methylmethansulphonate* (10 µg/ml for 3 hour treatment and 5 µg/ml for 24 hour treatment). In the presence of S-9 mix, *3-methylcholanthrene* (2.5 µg/ml) was used.

Media: RPMI 1640 (not spezified) was supplemented with 0.1% synperonic F68, 0.011% sodium pyruvate, 2 mM L-glutamine, 50 µg/ml gentamicin and buffered with 2 mg/ml sodium bicarbonate and this combination was referred to as R0p. R0p, supplemented with 10% HiDHS (not spezified), was used for general cell culture and was referred to at R10p. R10p, from which growing L5178Y cells had been removed, was used as conditioned medium. RPMI 1640 supplemented with 2 mM L-glutamine, 50 µg/ml gentamicin and buffered with 2 mg/ml sodium bicarbonate was referred to as R0. This medium was used during the treatment period only. RPMI 1640 supplemented with 0.02% synperonic F68, 0.011% sodium pyruvate, 2 mM L-glutamine, 50 µg/ml gentamicin, 30% HiDHS and buffered with 2 mg/ml sodium bicarbonate was referred to as R30p. R20p, which was used for Day_0 relative survival plating, consisted of a 50:50 mixture of R10p and R30p. Selective medium consisted of R10p containing 4 µg/ml TFT (not spezified).

S-9 fraction was prepared from a group of 8 male Sprague-Dawley derived rats stimulated by Aroclor 1254.

Preliminary toxicity testing: A cell suspension was prepared in a 1:1 mixture of R10p and conditioned media. The cell suspension was placed on a roller apparatus for 30 minutes, and then 3 ml aliquots of the suspension were dispensed into sterile universal tubes. R0 or S-9 mix (2 ml) was added to each culture. Cultures (one with and one without S-9 mix) were prepared for each concentration of test compound. For treatment in the absence of S-9 mix using a continuous treatment over 24 hours, a cell suspension was prepared in R10p. Test substance was diluted to provide serial concentrations that were then incorporated into the cell suspensions. Fifty microliters of test substance or solvent were added to each suspension. The final concentrations of the test substance in the culture medium were 4.69,

9.38, 18.75, 37.5, 75, 150, 300, 450 and 600µg/ml. Cultures were placed on the roller apparatus for 3 or 24 hours at 37 °C. The cells were then washed with R10p and resuspended in 20 ml of R10p and counted on a Coulter Counter. A series of dilutions was then prepared and the cell cultures were plated and incubated for at least 7 days. The original cell suspensions were transferred into pre-gassed roller bottles and rolled for 48 hours. Suspension growth was monitored by sampling at 24-hour intervals. Cell density was counted using an electronic particle counter. After the initial count at 24 hours after treatment, the cell density was adjusted using R10p. The number of colonies per plate was counted and the Day_0 relative survival was calculated. This estimate of toxicity was then used to determine the concentrations of test substance to be used in the main tests.

Due to excess toxicity, a second preliminary test in the absence of S-9 mix using both the 3 hour treatment period and a continuous treatment period for 24 hours was carried out. The final concentrations of the test substance in the culture medium were 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 20 µg/ml.

Main test**:** Cell suspensions were prepared as in the toxicity testing with minor modifications. The size of the aliquots of cell suspensions, the amount of medium, and the amount of test substance or solvent added to the tubes were double the size used in the toxicity testing. The number of cultures prepared was doubled, two with and two without S-9 mix were prepared for each concentration of test substance. Throughout the main tests, toxicity was measured in terms of Day_0 relative survival (RS), and not suspension growth or relative total growth. The cultures were treated with 0.05, 0.1, 0.5, 1, 1.5, 2, 3, 4 and 5 µg/ml in the absence of S-9 mix and 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15 and 20 µg/ml in the presence of S-9 mix. All cultures were returned to the roller apparatus for 3 hours.

The cells then were washed once, re-suspended and Day_0 relative survival (RS) was assessed. The remaining cell suspensions were transferred into pre-gassed roller bottles and rolled for 48 hours to allow for expression of the mutant phenotype. Suspension growth was monitored by sampling at 24 and 48 hours to assess growth in suspension. After 48 hours the cells were assessed for cloning efficiency (Day_2) and mutant frequency.

Cloning efficiency was assessed by plating in R10p. Mutant frequency was assessed by plating in selective medium. The plates were placed in a humidified incubator at 37° C in an atmosphere of 5% CO_2 in air.

As a negative result was obtained in the first test, a second test in the absence of S-9 mix using continuous treatment over 24 hours was carried out. The concentrations tested were 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml. A second test in the presence of S-9 was carried out using 3 hour treatment. The concentrations were 0.5, 1, 3, 5, 6, 7, 8, 9 and 10 µg/ml.

After plates were incubated for at least 7 days, for cloning efficiency, or 10-14 days, for mutant frequency, the number of empty wells was determined for each microtitre plate (P0). This figure was used to calculate cloning efficiency and mutant frequency.

Day_0 relative survival (RS) and Day_2 cloning efficiencies (CE) were calculated as follows:

P(0): number of empty wells/total wells

CE: InP(0)/number of cells per well

Cell count factor: Individual treated post-treatment cell count/ Mean control post-treatment cell count

Survival: CE x cell count factor

RS: Individual survival value x 10/Mean control survival value

Toxicity was expressed in terms of Day_0 relative survival and not suspension growth or relative total growth. The mutant frequency per 10^6 survivors was calculated as follows.

CE in selective medium/CE in non selective medium

Evaluation criteria:

The statistical significance of the data was analyzed by methods described by Robinson *et al.* (1989). Criteria for a response were:

The demonstration of a statistically significant increase in mutant frequency following treatment with the test substance.

Evidence of a dose relationship, over at least two dose levels, in any increases in mutant frequency.

Demonstration of reproducibility in any increases in mutant frequency.

The observed increases in mutant frequency must lie outside the historical control range with a corresponding Day_0 RS of not less than 10%.

The main test was done twice to assess reproducibility of responses.

Findings:

Preliminary toxicity: Treatment with 4.69 - 600 µg/ml fluazinam in the presence of S-9 mix resulted in a Day_0 relative survival over the range of 54 - 0% compared to the solvent controls. In the absence of S-9 mix, excess toxicity was observed after treatment with 4.69 - 600 µg/ml fluazinam with both the 3 hour and 24 hour treatment period and the results of this test are not reported. In the additional preliminary toxicity test with both a 3 hour and 24 hour treatment period, the resulting Day_0 relative survivals were 116 - 0% and 105 - 0%, respectively. Concentrations used in the main test were based on these data.

Main test – absence of S-9: Treatment with 0.05-5 µg/ml in Test 1, using a 3 hour treatment period, and 0.005-0.5 µg/ml in Test 2, using the continuous treatment period of 24 hours, in the absence of S-9 mix resulted in Day_0 relative survivals of 90-1% and 127-2%, respectively. Cultures treated with 1, 1.5, 2, 3 and 4 µg/ml in Test 1 and 0.02, 0.05, 0.1, 0.2 and 0.3 µg/ml in Test 2 were plated with and without TFT (selective agent) to permit measurement of the levels of cloning efficiency and induced mutation. The resulting Day_2 cloning efficiencies over this range were 87-20% in Test 1 and 103-62% in Test 2 relative to controls.

Statistically significant, dose-related increases in mutant frequency which were outside the historical control range were not observed in either test after treatment with IKF-1216. MMS, the positive control, induced highly significant increases in mutant frequency in both tests.

Main test – presence of S-9: Treatment of cells with 0.5-20 µg/ml in Test 1 and 0.5-10 µg/ml

in Test 2 resulted in Day_0 relative survivals of 97-0% and 107-3%, respectively. Cultures treated with 2.5, 5, 7.5, 10 and 12.5 µg/ml in Test 1 and 1, 3, 6, 7 and 9 µg/ml in Test 2 were plated with and without TFT (selective agent) to permit measurement of the levels of cloning efficiency and induced mutation. The resulting Day_2 cloning efficiencies over this range were 86 - 1% in Test 1 and 134 - 38% in Test 2 relative to controls.

In the presence of S-9 mix, an increase in mutant frequency was seen in the first test at an extremely toxic dose level of 12.5 µg/ml fluazinam (mean cell survival only 1 %). In concentrations of 2.5, 5, 7.5 and 10 µg/ml fluazinam, no increases in mutant frequency were observed. In the second test, concentrations of 1, 3, 6, 7 and 9 µg/ml fluazinam caused no increases in mutant frequency in the presence of S-9 mix. 3-Methylcholanthrene, the positive control, induced highly significant increases in the mutant frequency in both tests (table 6.4.1-1).

Table 6.4.1-1 Cytotoxicity and mutant frequency in mouse lymphoma cells (mean values)

Compound	conc. (µg/ml)		mean cell survival (% of control)		cloning efficiency [1] (%)		Mean mutant frequency per 10^6 survivors [2]	
	1st test	2nd	1st test	2nd	1st test	2nd	1st test	2nd
without S-9 mix								
DMSO	-	-	100	100	100	100	74	84
Fluazinam		0.005		97				
		0.01		100				
		0.02		127		89		77
	0.05	0.05	90	88		103		98
	0.1	0.1	89	63		90		108
		0.2		33		93		109
		0.3		8		62		264
		0.4		3				
	0.5	0.5	77	2				
	1.0		70		78		83	
	1.5		43		79		89	
	2.0		24		87		100	
	3.0		8		40		190	
	4.0		3		20		199	
	5.0		1					
Methylmethan-sulphonate	10	5.0	74	55	62	52	728**	1548**
with S-9 mix								
DMSO	-	-	100	100	100	100	96	136

Compound	conc. (µg/ml)		mean cell survival (% of control)		cloning efficiency [1] (%)		Mean mutant frequency per 10^6 survivors [2]	
	1st test	2nd	1st test	2nd	1st test	2nd	1st test	2nd
Fluazinam	0.5	0.5	96	107				
	1.0	1.0	97	92		134		102
	2.5		39		77		105	
		3.0		70		93		114
	5.0	5.0	62	47	86		105	
		6.0		32		76		191
		7.0		23		38		186
	7.5		21		67		153	
		8.0		14				
		9.0		15		111		225
	10.0	10.0	6	3	28		227	
	12.5		1		1		1174**	
	15.0		0					
	20.0		0					
Methyl-cholanthrene	2.5	2.5	96	76	43	93	1120**	808**

** ($p \leq 0.01$) significantly different from control
1) % cloning efficiency = total number of colonies on non-selective plates x 100/number of cells seeded (600)
2) total number of colonies on selective plates x 600/number of colonies on non-selective plates

Conclusions:

It is concluded that fluazinam did not demonstrate mutagenic potential in *in vitro* gene mutation assay.

Chromosomal aberration test of fluazinam technical using cultured mammalian cells

Reference: *Kajiwara Y.; 1988;* Report No.T-1663E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This study assessed the clastogenic potential of technical fluazinam (lot number 109, purity 95.3%, dissolved in DMSO) by means of *in vitro* chromosomal aberration tests using Chinese-hamster lung fibroblasts (CHL cells, obtained from the National Institute of Hygienic Sciences) in the absence or presence of metabolic activation (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). CHL cells preserved by freezing were defrosted and cultured (cell culture: 5 ml of 10 % NCS/MEM (Eagles minimum essential medium supplemented with 10 % newborn calf serum)). 3 days after incubation, a

subculture was made and the CHL cells in the logarithmic growth phase (stimulated to divide by treatment with phytohaem-agglutinin) were used for the test.

The concentrations of fluazinam tested were 1, 2 and 4 µg/ml without metabolic activation and 2.375, 4.75 and 9.5 µg/ml with S-9 mix, based on growth inhibition tests with fluazinam at concentrations of 0, 0.25, 0.5, 1, 2, 2.5, 4, 5, 7.5, 8, 10, 15, 20, 25 and 50 µg/ml (without metabolic activation) and 0, 0.5, 1, 2, 5, 8, 10, 15, 20 and 50 µg/ml (with metabolic activation).

Without S-9 mix, the two-day-old cultures were incubated for 24 and 48 hours at 37 ° C with the test substance. Cells which received S-9 mix were treated with the test material for 6 hours at 37 ° C. After S-9 mix including the test substance had been removed, the dishes were rinsed, placed in fresh media and incubated for further 18 hours.

2 hours prior to the end of the incubation, 0.1 µg/ml colcemid was added in order to prepare microscope slides of chromosomes.

Metaphase cells were then harvested and prepared for cytogenetic analysis.

Non-treatment and a solvent treatment group (DMSO) served as negative controls.

Mitomycin C (MMC, 0.05 and 0.025 µg/ml) and cyclophosphamide (CPA, 5 µg/ml) were used as positive controls for direct-method and metabolic activation, respectively.

Evaluation criteria:

The test substance was considered positive if the incidence of cells with aberrations was increased more than 10% in a dose-related manner, or if the incidence was reproducible for at least one of the test points.

Findings:

In the solvent treatment and non-treatment groups without metabolic activation, the incidences of cells with structural chromosomal aberrations including gaps were 0 % and 1 % respectively after 24 and 48 hour treatment. The positive control treated with MMC showed structural chromosomal aberrations at an incidence of 59 % after 24 hour treatment and 34 % after 48 hour treatment.

With metabolic activation, the incidences of cells with structural chromosomal aberrations including gaps were 0.5 % and 1 % for the non-treatment and solvent treatment groups, respectively. The positive control treated with CPA showed structural chromosomal aberrations at an incidence of 51.5 %.

After treatment with fluazinam, the mean percentage of cells with structural chromosomal aberrations including gaps was in a range between 0 % and 2 % with or without metabolic activation. These results indicate negative mutagenic activity of the test substance (table 6.4.1-2).

Table 6.4.1-2 Mean % of aberrant cells (including and excluding gaps)

Dose (µg/mL)	Without metabolic activation		With metabolic activation
	24 hours	48 hours	24 hours

	Excl. Gaps	Incl. gaps	Excl. gaps	Incl. gaps	Excl. gaps	Incl. gaps
1 µg/mL	0.5	0.5	0	0		
2 µg/mL	0.5	0.5	0	0		
2.375 µg/mL					1	1
4 µg/mL	1	1.5	1	1		
4.75 µg/mL					0	0.5
9.5 µg/mL					2	2
DMSO (solvent control)	0	0	0.5	1	1	1
Negative control	0.5	1	0	0	0	0.5
MMC (Mitomycin C)	58***	59***	29**	34**		
CPA (Cyclophosphamide)					50.5***	51.5***

** significantly different from controls at $p \leq 0.01$; *** significantly different from controls at $p \leq 0.001$

Conclusion:

It was concluded that fluazinam did not induce chromosomal aberrations in chinese hamster lung cells under both the metabolic activation and nonactivation conditions of this assay.

DNA repair test of fluazinam technical in Bacillus subtilis

Reference: *Ohtsuka M.; 1988;* Report No.T-1595E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of technical fluazinam (lot number 109 purity 95.3%) in the *Bacillus subtilis* [H17(rec^+) and M45(rec^-)]. Fluazinam was tested in the DNA repair assay (Spore method), at concentrations of 0.003, 0.01, 0.03, 0.1 and 0.3 µg/disk without metabolic activation (S-9 mix), and 0.3, 1, 3, 10 and 30 µg/disk with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). *B.subtilis* was incorporated into agar. Paper disks were soaked with 20 µl containing various amounts of fluazinam, placed on the agar and incubated for 24 hours at 37° C. For those plates tested with metabolic activation, the S-9 fraction was incorporated in the agar. The zone of growth inhibition was determined for each plate. DMSO was used as the solvent control. Positive control materials used were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) and 2-aminoanthracene (2-AA), and Kanamycin was used as a negative control.

Evaluation criteria:

The minimal inhibition concentrations, (MIC), MIC rec^+ and MIC rec^-, are obtained from regression analysis of the dose-response relationship. The test is considered positive if the index of DNA damage (MIC rec^+ / MIC rec^-) is 2 or higher.

Findings:

In case of no metabolic activation, growth of both bacterial strains was inhibited at a dose of 0.03 µg/disk and above. With metabolic activation, growth inhibition of both strains was noted at a dose of 1 µg/disk and above. There were no differences in the zones of inhibition between the strains with or without S9 mix. The index of DNA damage (MIC rec^+/MIC rec^-) was less than 2. The positive and negative controls gave the anticipated results.

Conclusion:

The results indicated that fluazinam was negative in the DNA repair test at the tested concentrations.

B.6.4.2 In vivo studies

IKF-1216 technical: Micronucleus test in mice

Reference: *Matsumoto K.; 1999;* Report No. IET 98-0139

Guideline: The study was conducted according to OECD Guideline 474 (1997), U.S. EPA Health Effects Guidelines (1991) and Japanese MAFF, 59 NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This study was conducted to assess the potential induction of micronuclei by fluazinam technical (Lot No. 8412-20, purity of 95.6%) in bone marrow cells of mice. In Micronucleus test I (time-course study), 5 male and 5 female mice were treated with a single oral dose of fluazinam (intragastric gavage) at a dose level of 2000 mg/kg bodyweight (a preliminary toxicity test had previously shown that a dose of 2000 mg/kg was tolerated; this level was therefore selected as an appropriate maximum for use in the micronucleus test.). Bone marrow smears were prepared at three sampling times, i.e., 24, 48 and 72 hours after administration and examined for the frequency of micronucleated polychromatic erythrocytes.

In Micronucleus test II (dose-response study), mice were treated per gavage with a single oral administration of fluazinam at dose levels of 500, 1000 and 2000 mg/kg bodyweight. The bone marrow smears were prepared once 24 hours after administration.

The negative control group received the vehicle, olive oil, and bone marrow smears were obtained from five male and five female animals 24, 48 and 72 hours after dosing.The positive control group was dosed with mitomycin C at 10 mg/kg bodyweight and bone marrow smears were prepared once 24 hours after administration.

One smear from each animal was examined using light microscopy. The frequencies of micronucleated polychromatic erythrocytes and the polychromatic erythrocyte ratios were analyzed. The frequencies of micronucleated polychromatic erythrocytes are shown as percentages of polychromatic erythrocytes with micronuclei among 2000 polychromatic erythrocytes. The polychromatic erythrocyte ratios as an indicator of hematopoesis are shown as percentages of polychromatic erythrocytes among 1000 erythrocytes scored.

Evaluation criteria:

The mean frequency of micronucleated polychromatic erythrocytes in the negative control group should be 0.3% or less. The mean frequency of micronucleated polychromatic erythrocytes in the positive control group should be 2.0% or more.

Unless there is a substantial difference in response between sexes, results for the two sexes are combined to facilitate interpretation and maximize the power of statistical analysis.

A positive response is indicated if statistically significant dose-response increases in the frequency of micronucleated polychromatic erythrocytes are observed.

Findings:

In the time course study (micronucleus test I), no adverse clinical signs were observed in treated and control mice for the duration of the test. There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes at any sampling time of the fluazinam group either for males and females separately or combined. In the positive control group (mitomycin C), an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes. Statistically significant differences in the polychromatic erythrocyte ratio were observed in the fluazinam group but not the positive control group at the 24 and 48 hour sampling times. When these values (55.9% and 50.5%) were compared to the value of the vehicle control group at the 72 hour sampling time (53.6%), there were no differences between these values.

In the dose response study (micronucleus test II), in the fluazinam treated groups loose stool or external genital region soiled fur was observed in nine male mice. These signs were not observed in the positive and negative control groups.

There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes at any dose of fluazinam either for males and females separately or combined. In the positive control group treated with mitomycin C, an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes. Statistically significant decreases in the polychromatic erythrocyte ratios were not observed at any dose level of fluazinam or in the positive control group treated with mitomycin C.

Table 6.4.2-1: Frequencies of micronucleated polychromatic erythrocytes (MNPCE/PCE) and polychromatic erythrocyte ratios (PCE/(PCE+NCE)

Sampling time	Treatment	Dose (mg/kg)	MNPCE/PCE (%) (mean)	PCE/(PCE+NCE) (%) (mean)
24 hours	Vehicle control	-	0.15 (test I) 0.16 (test II)	51.0 (test I) 53.6 (test II)
	Fluazinam	500	0.18	58.5
		1000	0.13	56.6
		2000	0.17 (test I) 0.15 (test II)	55.9*(test I) 51.5 (test II)
	Mitomycin C	10	3.30 (test I) 2.43 (test II)	47.3 (test I) 54.7 (test II)

Sampling time	Treatment	Dose (mg/kg)	MNPCE/PCE (%) (mean)	PCE/(PCE+NCE) (%) (mean)
48 hours	Vehicle control	-	0.14	59.0
	Fluazinam	2000	0.17	50.5*
72 hours	Vehicle control	-	0.14	53.6
	Fluazinam	2000	0.12	55.8

* significantly different vom vehicle control at $p \leq 0.05$ (Kastenbaum-Bowman and Wilcoxson's sum of ranks test)
MNPCE: micronucleated polychromatic erythrocytes
PCE: polychromatic erythrocytes
NCE: normochromatic erythrocytes

Conclusions:

From the results obtained, it is concluded that a single oral administration of fluazinam technical does not induce micronuclei in the bone marrow cells of ICR male and female mice under the conditions of this test.

B.6.4.3 Summary and overall conclusions of genotoxicity studies

Mutagenicity assays performed with fluazinam *in vitro* included gene mutation tests in bacteria (*S. typhimurium and E.coli*) and in mammalian cells (*mouse lymphoma*), a chromosomal aberration test in mammalian cells (Chinese hamster lung fibroblasts) and a DNA repair test in bacteria (Bacillus subtilis). Results from these studies showed that fluazinam did not induce gene mutation in any of the bacterial tester strains of *S. typhimurium* and *E.coli,* or gene mutation in mammalian cells in culture (mouse lymphoma). No potential for clastogenicity was observed in the in vitro chromosome aberration test in chinese hamster lung fibroblasts (CHL). There was also no induction for DNA damage observed in the DNA repair test with B.subtilis.
In the *in vivo micronucleus test* no induction of micronuclei by fluazinam in mouse bone marrow cells could be observed (table 6.4.3 -1).

Table 6.4.3-1: Summarised results of genotoxicity studies with fluazinam

Type of study	Test system	Dose range	Results	Reference
In vitro-studies				
Bacterial mutation assay	S. typhimurium (TA1535, TA1537, TA98 and TA100) and E. coli WP2uvrA/pKM101 (CM891)	0.005, 0.015, 0.050, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate	Negative	*Kitching J.;2000*
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0625 - 2 µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 - 500 µg/plate (with S-9 mix)	Negative	*Ohtsuka M.; 1988*

Type of study	Test system	Dose range	Results	Reference
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0313 - 1µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 - 500 µg/plate ((with S-9 mix)	Negative	*Ohtsuka M.; 1989*
Mammalian cell mutation assay	mouse lymphoma L5178Y cells	First test: 0.05 - 5 µg/ml (without S-9 mix); 0.5 - 20 µg/ml (with S-9 mix) Second test: 0.005 - 0.5 µg/ml (without S-9 mix); 0.5 - 10 µg/ml (with S-9 mix)	Negative	*Ransome S.; 2000*
Chromosomal aberration test	CHL	1 - 4 µg/ml (with S-9 mix); 2.375 - 9.5 µg/ml (without S-9 mix)	Negative	*Kajiwara Y.; 1988*
DNA repair test	bacillus subtilis	0.003 - 0.3 µg/disk (without S-9 mix), 0.3 – 30 µg/disk (with S-9 mix)	negative	*Ohtsuka M.; 1988*
In vivo-studies				
Micronucleus test	mouse bone marrow	single oral doses of 0, 500, 1000 and 2000 mg/kg bw	negative	*Matsumoto K.; 1999*

B.6.5 Long term toxicity and carcinogenicity (Annex IIA 5.5)

B.6.5.1 Oral studies in rats

B-1216: Potential Carcinogenicity and Chronic Toxicity Study in Dietary Administration to Rats for 104 Weeks:

Reference: *Mayfield R. et al; 1988;* Report No. ISK 8/87263

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.

The study is in compliance with GLP.

Material and method:

Five main groups of 50 male and 50 female rats and five satellite groups each consisting of 10 males and 10 females (strain: Sprague-Dawley; source: Charles River breeding laboratories, Portage, Michigan, USA) received diets containing 0, 1, 10, 100 and 1000 ppm fluazinam (batch 8412-20, purity 95.2 %), equivalent to 0, 0.04, 0.38, 3.82 and 40 mg/kg bw/d for males and 0, 0.05, 0.47, 4.87 and 53 mg/kg bw/d for females. Animals of the main group received the diet for 104 weeks, animals of the satellite groups were maintained for interim sacrifice after 52 weeks of treatment. Diets were prepared at fortnightly intervals; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed twice daily for clinical signs or reaction to treatment. A physical examination was done daily for the first 4 weeks of the study and afterwards once each

week. Food consumption was measured weekly, body weights were assessed on the first day of treatment, at weekly intervals thereafter and on the day of necropsy.
Ophthalmoscopic examinations were done before initiation and during weeks 26, 52, 78 and 101 on 10 males and 10 females from the control and high dose groups. Pupils were dilated with 0.5 % tropicamide, then the eyes were examined with an indirect ophthalmoscope.
Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC differential), clinical chemistry (glucose, urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT, AST, phospholipids, sodium, potassium, chloride, calcium, phosphor) and urinanalysis (appearance, volume, pH, specific gravity, glucose, bilirubin, protein, ketones, blood, urobilinogen, sediment) parameters were evaluated for 10 males and 10 females of each dosing group during weeks 12, 25, 51, 77 and 101 of treatment. A necropsy was done on each animal after 52 and 104 weeks of treatment, resp. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thyroid) were recorded. Histopathological examinations were performed on kidneys, liver, lungs, adrenals, aorta, brain, gastrointestinal tract, epididymides, eye and optic nerve, heart, lungs, marrow smear, lymph nodes, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, testes, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus (cervix and corpus) and vagina.
!n addition, a microscopic re-evaluation of brain tissue and cervical spinal cord from all control and high dosed rats of this study was conducted (Addendum 7). Slides were reviewed by the study pathologist and then subjected to a peer review by a second pathologist:

Findings:

General observations: An improved survival was apparent amongst treated groups in comparison to controls, particularly amongst rats receiving 1000ppm. Straw discolouration of the fur was noted in all high dosed animals from week 19 of the study. Females of the high dose group showed also an increased incidence of alopecia at the terminal sacrifice.
Food consumption of high dosed animals was lower than the concurrent control values, frequently attaining statistical significance.
Body weights and body weight gains of males and females receiving 1000 ppm were significantly lower than those of the respective control values (table 6.5.1-1).

Table 6.5.1-1: Mean body weight values (g) of rats treated with fluazinam

	Group 1	Group 2	Group 3	Group 4	Group 5

Weeks	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm
Males					
12-14	533	534	534	524	497**
25-27	631	629	629	611	573**
51-53	747	745	745	720	661**
77-79	822	777	798	768	689**
102-104	761	691	746	743	695
Body weight gain weeks 1-104	475	434	468	425	402
% control	-	91	98	89	85
Females					
12-14	299	295	293	291	261**
25-27	346	342	341	337	292**
51-53	452	440	430	427	358**
77-79	520	500	497	491	406**
102-104	551	529	485	525	417**
Body weight gain weeks 1-104	367	327	313	332	239
% control	-	89	85	90	65

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01 (William's t.)

Ophthalmoscopic examinations revealed no evidence of treatment-related changes in the high dose groups compared to controls.

Evaluation of hematological parameters revealed significantly lower packed cell volume, hemoglobin concentrations and erythrocyte counts in males and females from all treatment groups at weeks 13 and 26 compared to controls, although there was no strict dose-relationship and statistical significance was not always attained. At weeks 52 and 78 decreases for this parameters were observed in animals receiving 100 and 1000 ppm fluazinam, being more severe at week 52 than at week 78 and also more severe in males than in females. There were no obvious intergroup differences at week 102 (table 6.5.1-2).

Table 6.5.1-2: Relevant haematological findings (group mean values) in rats after 102 weeks of treatment with fluazinam

Parameter	Week	Group 1 0 ppm	Group 2 1 ppm	Group 3 10 ppm	Group 4 100 ppm	Group 5 1000 ppm
Males						
Packed Cell Volume %	13	50.8	49.2	47.9*	48.4*	49.7*
	26	48.1	46.6*	46.7*	46.7*	45.0**
	52	49.4	48.7	49.8	47.4*	45.8**

Parameter	Week	Group 1 0 ppm	Group 2 1 ppm	Group 3 10 ppm	Group 4 100 ppm	Group 5 1000 ppm
Males						
	78	46.2	46.1	46.8	44.5	43.5
	102	45.1	44.0	45.0	43.7	43.6
Hemoglobin g/dl	13	15.3	14.9	14.7*	14.8*	14.4**
	26	15.43	14.86	15.05	15.08	14.72**
	52	15.37	15.08	15.09	14.36**	13.59**
	78	14.3	14.0	14.2	13.3	12.7**
	102	13.75	13.57	13.75	13.26	13.09
Red Blood Cell $x10^6/mm^3$	13	7.40	7.45	7.45	7.38	7.46
	26	8.10	7.97	8.12	8.14	8.31
	52	8.52	8.60	8.59	7.99*	7.77**
	78	7.07	7.08	7.08	6.64	6.44*
	102	6.48	6.40	6.45	6.34	6.45
Females						
Packed Cell Volume %	13	50.0	48.8	47.4*	47.2**	46.2**
	26	45.0	44.7	43.4	43.2*	42.7**
	52	45.2	47.4	45.2	44.5	43.8
	78	44.0	45.1	43.7	43.3	41.4*
	102	42.6	43.9	46.4	44.4	44.3
Hemoglobin g/dl	13	15.4	14.9	14.6	14.5*	14.2**
	26	15.0	14.7	14.4*	14.7*	14.3**
	52	14.5	15.1	14.5	14.3	13.5*
	78	13.7	14.1	13.7	13.5	12.8
	102	13.39	13.86	14.42	13.70	13.83
Red Blood Cell $x10^6/mm^3$	13	7.12	6.88	6.94	6.82*	6.69**
	26	7.73	7.88	7.32	7.48	7.11**
	52	7.34	7.42	7.39	7.28	6.86*
	78	6.39	6.55	6.32	6.31	6.11
	102	5.95	6.18	6.55	6.25	6.28

*: Significant difference from control, $p<0.05$; **: significant difference from control, $p<0.01$ (William's test)

Blood chemistry examination revealed elevated cholesterol levels in both sexes of the high dose group and also in males of the 100 ppm group throughout the study and in females at week 52 compared to controls. Statistically significant increases in albumin and decreases in globulin were revealed in animals of the high dose group during week 13, in males also during week 26 and 52 and in males receiving 100 or 1000 ppm during week 102. ALT and AST levels were significantly elevated in females of the high dose group during week 52

compared to controls (table 6.5.1-3).

Table 6.5.1-3: Relevant clinical chemistry findings (group mean values) in rats after 102 weeks of treatment with fluazinam

Parameter	Week	Group 1 0 ppm	Group 2 1 ppm	Group 3 10 ppm	Group 4 100 ppm	Group 5 1000 ppm
Males						
Cholesterol mg%	13	61	56	59	69	73
	26	56	55	58	70	78*
	52	72	76	83	104*	95*
	78	101	105	107	125	130
	102	135	116	159	162	142
Albumin g/dl	13	3.9	3.7	3.8	3.9	4.1*
	26	3.6	3.7	3.6	3.6	3.9**
	52	4.0	4.0*	4.0	4.0	4.2*
	78	4.0	3.9	4.2	4.0	4.1
	102	3.6	3.7	3.7	3.8*	3.9**
Globulin g/dl	13	3.4	3.3	3.2	3.1	3.1*
	26	3.8	3.7	3.5	3.6	3.4*
	52	3.4	3.3	3.2	3.3	3.1**
	78	3.6	3.6	3.3	3.6	3.5
	102	3.8	3.7	3.4	3.3*	3.6*
ALT mU/ml	13	23	25	26	24	21
	26	18	22	20	21	19
	52	28	28	33	30	27
	78	27	26	34	26	32
	102	19	27	27	22	23
AST mU/ml	13	53	58	60	58	60
	26	42	44	43	46	48*
	52	58	55	59	58	57
	78	61	53	59	51	64
	102	44	70	57	46	56
Females						
Cholesterol mg%	13	76	79	68	76	94**
	26	77	88	74	82	96
	52	86	87	87	123*	130*
	78	98	90	92	145	153*
	102	101	107	116	129	217*
Albumin	13	4.3	4.3	4.4	4.3	4.6*

Parameter	Week	Group 1 0 ppm	Group 2 1 ppm	Group 3 10 ppm	Group 4 100 ppm	Group 5 1000 ppm
g/dl	26	4.3	4.7	4.4	4.6	4.5
	52	4.8	4.9	4.9	4.7	4.9
	78	4.5	4.5	4.6	4.4	4.4
	102	4.3	4.4	4.5	4.3	4.4
Globulin g/dl	13	3.3	3.0*	3.1*	3.0*	3.1*
	26	3.5	3.3	3.3	3.3	3.3
	52	3.2	3.1	3.2	3.3	3.3
	78	3.4	3.4	3.6	3.2	3.5
	102	3.7	3.4	3.5	3.3*	3.4*
ALT mU/ml	13	25	23	26	18	18
	26	20	30	22	22	21
	52	21	19	23	22	32*
	78	25	23	50	22	37
	102	28	31	32	37	54
AST mU/ml	13	57	63	63	54	54
	26	55	55	54	52	52
	52	44	42	44	48	66**
	78	55	47	49	46	62
	102	59	57	46	64	77

*: Significant difference from control, p<0.05 ; **: significant difference from control, p<0.01 (William's test)

Urinanalysis: there was no evidence of treatment-related changes at any dose group when compared to control animals.

Organ weight analyses: At the interim kill after 52 weeks of treatment, statistically significant higher liver weights, adjusted for bodyweight, were observed in both sexes receiving 1000 ppm and also in females receiving 100 ppm compared to controls. Thyroid weights were elevated in both sexes of the 1000 ppm-group, although statistical significance was attained in males only. At the terminal kill after 104 weeks of treatment, a statistically significant increase in liver weights was observed in females at the highest dose level, males of this dose group and females of the 100 ppm-group showed only slightly increased liver weights compared to controls. Thyroid weights were statistically significantly increased in males of the 1000 ppm-group only (table 6.5.1-4).

Table 6.5.1-4: Body and organ weights (g) of rats after 52 and 104 weeks of treatment with fluazinam (group mean values)

Group	Dose ppm	Body weight (g)		Thyroid weight (mg)		Liver weight (g)	
		M	F	M	F	M	F
Interim							
1	0	757	491	29.6	22.2	27.7	15.8
2	1	790	484	31.7	23.1	29.7	16.4
3	10	741	452	27.2	21.2	26.2	15.7
4	100	731	440	31.0	24.0	26.7	18.3*
5	1000	663	387**	42.4**	27.4	35.2*	19.2**
Terminal							
1	0	738	542	45.7	39.5	27.9	18.5
2	1	700	502	42.7	30.9	27.5	17.5
3	10	716	480	43.9	34.2	28.0	18.5
4	100	698	499	44.2	38.3	27.0	20.4
5	1000	678	413**	52.1	38.1	29.9	23.0**

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01 (William's Test)

At necropsy, macroscopic examinations revealed yellow staining of the fur in both sexes of the highest dose group at the interim sacrifice and also at the terminal kill after 104 weeks of treatment. Organ changes were observed at the terminal kill only and consisted of liver lesions (enlargement and pale areas in the 1000 ppm groups), testicular lesions (flaccid in the highest dose group and white subtunical striae in the 100 and 1000 ppm-groups). Enlarged thyroids were observed in males of the highest dose group.

Histopathological examinations at the interim sacrifice after 52 weeks of treatment showed centrilobular hepatocyte vacuolation in animals receiving 1000 ppm, especially in males, compared to controls. This change appeared to be associated with deposits of centrilobular fat.

Pancreatic acinar epithelial vacuolation was seen amongst females receiving 1000 ppm and exocrine degranulation of the pancreas in females receiving 100 and 1000 ppm.

At the terminal sacrifice after 104 weeks of treatment, lungs of males and females receiving 1000 ppm and also of females receiving 100 ppm showed adenomatosis and/or pneumonitis and an increased incidence of alveolar epithelialisation.

An increased incidence of exocrine pancreatic atrophy was observed in the 1000 ppm group in both sexes and in females receiving 100 ppm. Pancreatic exocrine cellular vacuolation associated with fat deposition was seen amongst females receiving 1000 ppm.

In the liver, eosinophilic hepatocytes were recorded in both sexes of the 1000 ppm-group and also in females receiving 100 ppm fluazinam. In both sexes receiving 1000 ppm a centrilobular hepatocyte vacuolation was observed. In females only receiving 1000 ppm there was also an increase of centrilobular hepatocyte necrosis, compared with centrilobular

fat deposition. In both sexes receiving 100 and 1000 ppm there was a dilatation of centrilobular liver sinusoids observed. Male and female livers of the highest dose group showed also an increase of bile-duct hyperplasia.

Lymph nodes of females of the 1000 ppm group showed sinus histiocytosis.

An increased incidence of testicular atrophy was observed at 100 and 1000 ppm and also an increase of spermatocele granuloma at 1000 ppm (table 6.5.1-5).

There was no effect of treatment on the incidence or degree of vacuolation of white matter in the brain or spinal cord of rats of the high dose group (1000 ppm) compared to controls.

Table 6.5.1-5: Incidences (%) of microscopic findings of male and female rats after 104 weeks of treatment with fluazinam (group mean values)

	0 ppm		1 ppm		10 ppm		100 ppm		1000 ppm	
Sex	m	f	m	f	m	f	m	f	m	f
Lungs										
Adenomatosis	0	2	0	0	0	0	0	0	8	0
Pneumonitis	2	2	2	0	2	2	6	8	16	10
Epithelialisation	0	0	0	0	2	2	0	8	34	30
Pancreas										
Exocrine atrophy	14	6	22	12	18	16	26	36	38	26
Exocrine cellular vacuolation	0	0	2	0	2	2	0	6	2	56
Liver										
Eosinophilic hepatocytes	18	8	12	18	18	18	12	24	42	74
Centrilobular vacuolation	14	36	20	24	12	20	28	46	78	76
Centrilobular necrosis	2	2	2	0	0	2	0	2	0	14
Dilatation of liver sinusoids	0	2	4	0	2	2	10	20	14	56
bile-duct hyperplasia	42	30	30	28	38	28	34	40	62	76
Lymph nodes										
Sinus histiocytosis	16	8	10	10	8	16	8	6	14	36
Testes										
Atrophy	14	-	30	-	18	-	38	-	40	-
Spermatocele granuloma	0	-	2	-	2	-	0	-	10	-

Conclusion:

No treatment-related effects were seen on the spontaneous tumor profile in rats at any dose level. Treatment-related non-neoplastic effects were seen at dose levels of 100 and 1000 ppm in livers, lungs, pancreas, lymph nodes and testes. In this study, the NOAEL for

fluazinam fed to rats for 104 weeks was 10 ppm, 0.38 mg/kg bw/d for males and 0.47 mg/kg bw/d for females.

B-1216: Toxicity to Rats by Dietary Administration for 2 Years:

Reference: *Chambers P. R. et al; 1993;* Report No. ISK 43/920649

Guideline: The study was conducted according to Japanese MAFF Test Guidelines (1985), U.S. EPA Guidelines and OECD Guideline No. 452 and is in compliance with GLP. The study is considered acceptable.

Material and method:

Groups of 25 male and 25 female rats (strain: Sprague-Dawley; source: Charles River breeding laboratories, Portage, Michigan, USA) received diets containing 0, 25, 50 and 100 ppm fluazinam (batch 8412-20, purity 95.2 %), equivalent to 0, 1.0, 1.9 and 3.9 mg/kg bw/d for males and 0, 1.2, 2.4 and 4.9 mg/kg bw/d for females, for 104 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed once daily for clinical signs or reaction to treatment. A detailed palpation of each rat was performed weekly in order to record the date of appearance, location and dimension of all new palpable masses. Food consumption and body weights were measured weekly. Ophthalmoscopic examinations were done before initiation and during weeks 13, 52 and 103 in animals from the control and high dose groups. Pupils were dilated with 0.5 % tropicamide, then the eyes were examined with an indirect ophthalmoscope. Hematology (hematocrit, hemoglobin, PT, RBC, MCV, MCH, MCHC, platelet count, reticulocyte count, PCV, WBC total and differential), clinical chemistry (glucose, urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, cholesterol, AP, ALT, AST, CPK, phospholipids, sodium, potassium, chloride, calcium, phosphor) and urinanalysis (appearance, volume, pH, specific gravity, glucose, bilirubin, protein, ketones, blood, urobilinogen, sediment) parameters were evaluated for 10 males and 10 females of each dosing group during weeks 13, 26, 52 and 78 and at termination of the study. A necropsy was done on each animal after 104 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, kidneys, liver, spleen, testes with epididymides) were recorded. Tissues required for microscopic examination were kidneys, liver, lungs, adrenals, aorta, brain, gastrointestinal tract, epididymides, eyes, heart, lymph nodes, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum (bone and marrow) , testes, thymus, thyroid with parathyroid, trachea, urinary bladder and uterus (cervix and corpus). In animals of the control and high dose groups and in all animals dying or killed in extremis during the study, these tissues were examined histopathologically. From animals of the intermediate and low dose group, lungs, liver, kidneys, spleen, pancreas, testes and epididymides were examined histopathologically.

Findings:

General observations: there were no clinical signs indicative of a reaction to treatment.

Food consumption of treated groups was compareable to the concurrent control values.

There was a mean body weight loss beginning about week 75 in high dose females which resulted in a statistically significant lower body weight gain of females of this group at the end of the treatment period compared to the respective control values (table 6.5.1-6).

Table 6.5.1-6: Mean body weight (g) of rats treated with fluazinam

Weeks	Group 1 0 ppm	Group 2 25 ppm	Group 3 50 ppm	Group 4 100 ppm
Males				
0 – 75	712	668	642	634
% of control	-	94	90	89
0 – 104	573	602	643	560
% of control	-	105	112	98
Females				
0 – 75	401	378	406	390
% of control	-	94	101	97
0 – 104	462	417	450	356*
% of control	-	90	97	77

*: significantly different from controls at p<0.05

Ophthalmoscopic examinations revealed no evidence of treatment-related changes in the high dose groups compared to controls.

Evaluation of haematological and blood chemistry parameters and urinanalysis: revealed no evidence of treatment-related changes at any dose group when compared to control animals.

Organ weight analyses: At the terminal kill after 104 weeks of treatment, a statistically significant increase in relative liver weights was observed in females at the highest dose level of 100 ppm, males of this dose group showed only slightly increased liver weights compared to controls. Relative testes and epididymides weights were statistically significantly increased in males of the 100 ppm-group (table 6.5.1-7).

Table 6.5.1-7: Body and organ weights (g) of rats after 104 weeks of treatment with fluazinam (group mean values)

Group	Dose ppm	Body weight (g)		Liver weight (g)		Testes+ Epididymides weight (g)
		M	F	M	F	M
1	0	739	586	27.6	19.3	4.38
2	25	770	539	28.8	20.2	4.75
3	50	806	574	27.2	21.0	4.48
4	100	722	479	31.2	23.9**	5.12**

**: significantly different from controls at p<0.01 (William's Test)

At terminal necropsy, there were no treatment-related macroscopic changes noted. A greater number of high dose males dying during the study had small and/or flaccid testes compared to controls, but no increased incidence was observed in males surviving to termination.
Histopathological examinations revealed no evidence of a treatment-related effect on the number or distribution of tumours observed in decedent and terminal animals.
Adenomatosis in the lungs was recorded in two males and one female receiving 50 ppm and in one female receiving 100 ppm. Alveolar epithelialisation was found in one male of the 25 ppm group, in two females of the 50 ppm-group and in one male and three females of the 100 ppm-group. Statistical significance was not reached.
An increased incidence of exocrine pancreatic atrophy, but not statistically significant, was observed in females receiving 50 and 100 ppm.
In the liver, an increased incidence of foci or areas of eosinophilic hepatocytes were recorded in males and females of the 100 ppm group compared to controls. However, the highest incidence of this finding was found in 25 ppm group males and non of the above reached statistical significance.
An increased incidence of marked testicular atrophy was observed in animals of the 100 ppm group compared to controls (table 6.5.1-8).

Table 6.5.1-8: Selected Pathology Findings (%) in Rats after 104 weeks of treatment with fluazinam

	Group 1 0 ppm		Group 2 25 ppm		Group 3 50 ppm		Group 4 100 ppm	
Finding	m	f	m	f	M	f	m	f
Lungs:								
Adenomatous hyperplasia	0	0	0	0	8	4	0	4
Alveolar epithelialization	0	0	4	0	0	8	4	12
Liver:								
Foci/areas of eosinophilic hepatocytes	44	32	68	44	44	56	60	48
Pancreas:								
Exocrine acinar atrophy	48	32	16	28	44	56	36	48
Testes:								
Tubular atrophy (total)	64		64		44		64	
Tubular atrophy (marked)	24		20		16		36	

Conclusion:

In this study, treatment-related changes were manifest at 100 ppm and resulted in slightly increased liver, testes and epididymides weights and macroscopically in a higher incidence of small/flaccid testes. Microscopically, evidence of toxicity was observed as an increased

incidence of marked testicular atrophy in males of the 100 ppm group. There was no evidence of any tumorigenic potential in male or female rats at any of the treatment levels investigated.

A dose level of 50 ppm fluazinam, equivalent to 1.9 mg/kg bw/d for males and 2.4 mg/kg bw/d for females, was considered as NOAEL in this study.

B.6.5.2 Oral studies in mice

B-1216: Potential Carcinogenicity Study in Dietary Administration to Mice for 104 Weeks:

Reference: *Mayfield R. et al; 1988;* Report No. ISK 9/87264

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.

The study is in compliance with GLP.

Material and method:

6 groups (including 2 control groups) of 52 male and 52 female mice (strain: CD-1; source: Charles River, Kent, U.K.) received diets containing 0, 1, 10, 100 and 1000 ppm fluazinam (Lot 8412-20; purity 95.3 %), equivalent to 0, 0.12, 1.12, 10.72 and 107 mg/kg bw/d for males and 0, 0.11, 1.16, 11.72 and 117 mg/kg bw/d for females, for 104 weeks. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were housed 4 to a cage (According to sex) and observed twice daily for clinical signs or reaction to treatment. A physical examination was done daily for the first 4 weeks of the study and afterwards once each week. A detailed palpation of each mouse was performed weekly in order to record the date of appearance, location and dimension of all new palpable masses. Food consumption was measured weekly, body weights were assessed one week before commencement, on the first day of treatment and once a week thereafter. Hematology parameters (Hb, RBC, MCV, MCH, MCHC, platelet count, PCV, WBC (total and differential) were evaluated for one male and one female of each cage including controls during weeks 26, 52, 78 and 104 of treatment. A necropsy was done on each animal that died and on all surviving animals after 104 weeks of treatment. At necropsy, each animal was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, ovaries, testes and spleen) were recorded. Histopathological examinations were performed on kidneys, liver, lungs, adrenals, aorta, brain, gastrointestinal tract, epididymides, eye and optic nerve, heart, marrow smear, lymph nodes, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus (cervix and corpus) and vagina.

!n addition, a microscopic re-evaluation of brain tissue and cervical spinal cord from all

animals of the study including controls was conducted (Addendum 8).

Findings:

General observations: There were no treatment-related clinical signs.

Body weight, body weight gains and food consumption were comparable to the respective control values.

Evaluation of hematological parameters revealed no differences from control values which were considered to be related to treatment with fluazinam.

Organ weight analysis after 104 weeks of treatment revealed statistically significant higher liver weights when adjusted for body weight in males and females of the 1000 ppm dose groups and also in females of the 100 ppm dose group compared to controls (table 6.5.2-1).

Table 6.5.2-1: Body and liver weights of mice treated with fluazinam for 104 weeks

	Male		Female	
Dose (ppm)	Body weight (g)	Liver (g)	Body weight (g)	Liver (g)
0[a]	43.49	2.607	35.77	1.745
1	41.82	2.923	36.74	1.817
10	42.38	3.056	36.51	1.760
100	44.07	2.523	37.21	2.008**
1000	43.03	3.789**	36.65	2.264**

[a]: two control groups combined; **: significantly different from controls at p<0.01 (William's Test)

At necropsy, macroscopic examinations revealed increased incidences of liver lesions consisting of masses, surface irregular or pitted, pale liver areas, pallor and accentuated lobular markings in high dosed males and a greater number of surface irregularities or pitting in females of this dose group compared to controls.

Histopathological examinations revealed a higher incidence of basophilic or eosinophilic hepatocytes in male mice of the 1000 ppm-group compared to controls. A higher incidence of granulomatous hepatitis and aggregates of brown pigmented macrophages were noted in high and mid-dose males and high dosed females (table 6.5.2-2).

Table 6.5.2-2: Incidences of non-neoplastic microscopic findings in mice after 104 weeks of treatment with fluazinam

Ppm	**0**	**0**	**1**	**10**	**100**	**1000**
Males						
Brown pigmented macrophages	9	5	6	5	14	32
basophilic hepatocytes	6	6	5	7	7	20
eosinophilic hepatocytes	5	2	4	3	3	6

Ppm	0	0	1	10	100	1000
granulomatous hepatitis	10	5	10	7	12	15
Females						
Brown pigmented macrophages	12	4	21	11	20	26
basophilic hepatocytes	1	4	3	2	2	2
eosinophilic hepatocytes	1	1	2	2	2	2
granulomatous hepatitis	11	4	10	5	5	15
Total number examined	**52**	**52**	**52**	**52**	**52**	**52**

Liver cell tumours (adenomas and carcinomas) were observed in a greater number of male mice receiving 1000 ppm compared to controls, statistically significant for adenomas. The testing facility`s (Huntingdon Research Centre Ltd.) historical control data for liver tumours in this strain of mice showed incidence in nine studies ranging from 3.8 to 26.9 % for adenomas and 11.5 to 38.5 % for carcinomas (tables 6.5.2-3 and 6.5.2-4).

Table 6.5.2-3: Incidences of liver cell tumours in male mice after 104 weeks of treatment with fluazinam

Ppm	0	0	1	10	100	1000
Number of mice examined	52	52	52	52	52	52
Adenoma (%)	6 (12)	9 (17)	12 (23)	9 (17)	7 (13)	17 (33)*
Carcinoma (%)	9 (17)	9 (17)	8 (15)	7 (13)	7 (13)	17 (33)

*: significantly different from controls at p<0.05 (William's Test)

Table 6.5.2-4: Huntingdon Research Centre Ltd. historical control data for liver tumours in male mice for the years 1981 – 1983

	Study number								
	1	2	3	4	5	6	7	8	9
Adenoma (%)	2 (3.8)	13 (12.5)	15 (14.4)	6 (11.5)	16 (18.2)	11 (21.2)	12 (23.1)	14 (26.9)	9 (17.3)
Carcinoma (%)	12 (23.1)	25 (24)	17 (16.3)	14 (26.9)	25 (28.4)	9 (17.3)	6 (11.5)	10 (19.2)	20 (38.5)
Number of mice examined	52	104	104	52	88	52	52	52	52

A statistically significant increase of vacuolation of white matter in the brain was seen in both

sexes of the high dose groups. In males, there were increased total incidences and also increased incidences of the minimal grade of vacuolation in the brain, compared to both control groups. In females of this dose group, there were increased incidences of minimal and moderate white matter vacuolation in the brain, compared to both control groups (although there was no increase in the total incidence). No treatment-related effects were seen in the brain of mice receiving 1, 10 or 100 ppm.

Table 6.5.2-5: Incidences of vacuolation of white matter in the brain of male mice after 104 weeks of treatment with fluazinam

Dosage level (ppm)		**0**	**0**	**1**	**10**	**100**	**1000**
Cerebrum							
No abnormalities detected		14	19	9	12	18	5
Vacuolation of white matter	Total	38	33	43##	40	34	47*##
	Trace	38	33	43	40	33	32
	Minimal	0	0	0	0	1	15
Cerebellum/pons/medulla							
No abnormalities detected		13	14	9	11	10	3
Vacuolation of white matter	Total	39	38	43	41	42	49**##
	Trace	25	32	34	28	28	27
	Minimal	14	6	9	13	14	22
Spinal cord							
No abnormalities detected		15	14	13	19	12	7
Vacuolation of white matter	Total	37	38	39	33	40	45*
	Trace	23	32	29	26	26	34
	Minimal	14	6	10	7	14	11
Number examined		52	52	52	52	52	52

significantly different from control group 1, *: p<0.05, **: p<0.01

significantly different from control group 2, ##: p<0.01 (Fisher's Exact Test)

Table 6.5.2-6: Incidences of vacuolation of white matter in the brain of female mice after 104 weeks of treatment with fluazinam

Dosage level (ppm)		0	0	1	10	100	1000
Cerebrum							
No abnormalities detected		4	6	4	1	2	1
Vacuolation of white matter	Total	48	46	48	51	50	51
	Trace	44	41	39	43	45	23
	Minimal	4	5	9	8	5	25
	Moderate	0	0	0	0	0	3
Cerebellum/pons/medulla							
No abnormalities detected		2	6	3	1	0	0
Vacuolation of white matter	Total	50	46	49	51	52#	52#
	Trace	14	22	25	23	27	19
	Minimal	35	23	24	28	24	27
	Moderate	1	1	0	0	1	6
Spinal cord							
No abnormalities detected		2	8	7	3	1	9
Vacuolation of white matter	Total	50	44	45	49	51#	43
	Trace	17	31	27	31	37	31
	Minimal	33	13	18	18	14	12
Number examined		52	52	52	52	52	52

significantly different from control group 2, #: $p<0.05$ (Fisher's Exact Test)

Conclusion:

Dietary administration of fluazinam at dose levels of 1, 10, 100 and 1000 ppm for 104 weeks resulted in treatment related changes in the liver in mice of high and mid dose groups (basophilic or eosinophilic hepatocytes in male mice of the 1000 ppm-group, granulomatous hepatitis and aggregates of brown pigmented macrophages in high and mid-dose males and high dosed females) and a statistically significant increase of vacuolation of white matter in the brain in both sexes of the high dose groups. Liver cell tumours were observed in a greater number of male mice of the high dose group, reaching an incidence both adenomas and carcinomas of 33 %. The historical control data for liver tumours in nine studies carried out at Huntingdon Research Centre Ltd. in the years 1981 – 1983 showed incidences of adenomas in the range of 3.8 to 26.9 % and carcinomas in the range of 11.5 to 38.5 %. Thus

the incidence of liver tumours were within the range of the historical control data. 10 ppm, equivalent to 1.14 mg/kg TG/d, were considered to be the NOAEL in this study.

Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice:

Reference: *Chambers P. R. et al; 1998;* Report No. ISK 50/950671 and Addendum 1

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.

The study is in compliance with GLP.

Material and method:

4 groups of 50 male and 50 female mice (strain: CD-1; source: Charles River, Michigan, USA) received diets containing 0, 1000, 3000 and 7000 ppm fluazinam (Lot 1030/91; purity 97 %), equivalent to 0, 126, 377 and 964 mg/kg bw for males and 0, 162, 453 and 1185 mg/kg bw for females for 104 (males) and 97 (females) weeks, respectively. Two satellite groups (males consisting of 20, females of 18 animals per group) were treated at 0 and 7000 ppm for 78 weeks and then subjected to a histopathological examination of the liver to determine when the main study would be terminated. Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

Animals were observed once daily for clinical signs or reaction to treatment. A physical examination was done once each week. Food consumption and body weights were measured weekly. White blood cell counts were evaluated during weeks 52, 78, 97 (females only) and 104 (males only).

The study was continued until the 75 % mortality point was reached in the females in week 97 and the males had completed 104 weeks of treatment.

At necropsy, each animal (including those of the satellite groups) was subjected to a detailed gross pathology examination and the weights of selected organs (adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes and epididymides) were recorded. Tissues required for microscopic examination were kidneys, liver, lungs, adrenals, aorta, brain, gastrointestinal tract, epididymides, eyes, heart, lymph nodes, oesophagus, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum (bone and marrow) , testes, thymus, thyroid with parathyroid, trachea, urinary bladder, uterus (cervix and corpus) and vagina. In animals of the control and high dose groups and in all animals dying or killed in extremis during the study, these tissues were examined histopathologically. From animals of the intermediate and low dose groups, lungs, liver, kidneys and any macroscopically abnormal tissue were examined histopathologically.

Findings:

General observations: Mortality was statistically significantly increased in high dosed females compared to controls (table 6.5.2-7). Due to this high mortality, females of all dose groups including controls were terminated after 97 weeks of treatment. Males were continued on

study through 104 weeks.

Table 6.5.2-7: Incidence and Percentage of mortality

Weeks	Mortality	0 ppm	1000 ppm	3000 ppm	7000 ppm
Males					
1 – 104	Incidence	32	24	29	34
	% mortality	64	48	58	68
Females					
1 – 97	Incidence	21	24	23	37*
	% mortality	42	48	46	74

*: significantly different from control: p<0.003 (logrank methods, Mantel 1966)

Body weight gain for males receiving 7000 ppm was slightly reduced compared to controls.
Food consumption was compareable to the respective control values for all treated male and female dose groups.
Hematology: Differential white cell counts revealed no differences from control values which were considered to be related to treatment with fluazinam.
Organ weight analysis revealed statistically significant higher liver weights when adjusted for body weight in males and females of all dosing groups sacrificed in weeks 79, 98 (females) and 105 (males) compared to controls. Brain weight analysis showed also statistical significant higher values for both sexes receiving 7000 ppm (sacrificed in week 98 and 105 respectively). Females of the high dose group showed also statistically significant higher adrenal weights compared to controls (table 6.5.2-8).

Table 6.5.2-8: Organ weights of mice treated with fluazinam

Tissue	Week	Males (ppm)				Females (ppm)			
		0	1000	3000	7000	0	1000	3000	7000
Liver (g)	79	2.44	-	-	4.02*	1.67	-	-	3.17**
	105/98	2.09	3.22**	4.46**	5.90**	1.84	2.23**	2.67**	3.85**
Brain (g)	79	0.490	-	-	0.489	0.497	-	-	0.492
	105/98	0.485	0.482	0.487	0.524**	0.507	0.491	0.500	0.544**
Adrenal (mg)	79	5.2	-	-	5.8	9.1	-	-	10.0
	105/98	6.3	6.5	7.3	7.9	10.1	9.3	9.9	11.9*

*: significantly different from controls at p<0.05; **: significantly different from controls at p<0.01

At necropsy, macroscopic examinations revealed swelling of the brain in 2/50 males and 3/50 females of the 7000 ppm group and in 1/50 females of the 3000 ppm group compared to none in controls. Liver enlargement, liver masses, raised/pale/dark areas, pallor, accentuated lobular markings and brown discoloration were noted in both sexes of the 7000 ppm and 3000 ppm groups. Irregular surface depressions in males and pitting of the liver in females were also noted among animals receiving 7000 ppm. Pallor, accentuated lobular markings and brown discoloration were also noted among some males receiving 1000 ppm.
Histopathological examinations revealed increased incidences of altered hepatocyte foci (basophilic and eosinophilic), hepatocyte enlargement, focal swollen/vacuolated hepatocytes, aggregates of macrophages containing brown pigment and parenchymal inflammatory cells in all treated groups.
A higher incidence of hepatocellular adenomas in males receiving 3000 ppm was noted compared to controls (table 6.5.2-9).

Table 6.5.2-9: Incidence of hepatocellular tumors in male mice (expressed in percentages)

Dosage level, ppm	0	1000	3000	7000
Hepatocellular adenoma	16	24	40**	28
Hepatocellular carcinoma	2	6	6	8
Number of mice examined	50	50	50	50

**: significantly different from control: p<0.01

Historical control data of Huntingdon Research Centre Ltd. for liver tumours in this strain of mice with study duration of 80 – 96 weeks showed incidences in twelve studies ranging from 8 to 34 % for adenomas and 1.8 to 16 % for carcinomas (table 6.5.9-10).

Table 6.5.2-10: Huntingdon Research Centre Ltd. historical control data for liver tumours in male mice for the years 1991 – 1993 (expressed in percentages)

	Study number											
	1	2	3	4	5	6	7	8	9	10	11	12
Adenoma	8	10.7	19.6	8	16	14	16	22	14	12	16	34
Carcinoma	8	8	8.9	10	1.8	12	6	4	4	12	6	16
Number of mice examined	50	56	56	50	50	50	50	50	50	50	50	50
Duration of study (weeks)	92	80	80	80	81	92	92	80	83	83	96	80

A statistically significant increase of vacuolation of white matter in the brain and cervical spinal cord was seen in a large number of animals receiving 3000 and 7000 ppm and in few

animals receiving 1000 ppm (tables 6.5.2-11 and 6.5.2-12).

Table 6.5.2-11: Incidences of vacuolation of white matter in the brain of male mice after 104 weeks of treatment with fluazinam

Dosage level (ppm)		0	1000	3000	7000
Cerebrum					
No abnormalities detected		26	9*	0**	1**
Vacuolation of white matter	Total	24	41*	50**	49**
	Trace	24	40*	24**	6
	Minimal	0	1	20**	21**
	Moderate	0	0	6*	12**
	Marked	0	0	0	10**
Cerebellum/pons/medulla					
No abnormalities detected		26	10**	5**	1**
Vacuolation of white matter	Total	24	40**	45**	48**
	Trace	22	35	22**	13
	Minimal	2	4	16**	15**
	Moderate	0	1	7*	10*
	Marked	0	0	0	10**
Spinal cord					
No abnormalities detected		32	26*	13**	4**
Vacuolation of white matter	Total	18	24*	37**	46**
	Trace	18	24*	30	17
	Minimal	0	0	6*	14**
	Moderate	0	0	1	10**
	Marked	0	0	0	5
Number examined		50	50	50	50

*: significantly different from control: p<0.05; **: significantly different from control: p<0.01 (Fisher's Exact Test)

Table 6.5.2-12: Incidences of vacuolation of white matter in the brain of female mice after 97 weeks of treatment with fluazinam

Dosage level (ppm)		0	1000	3000	7000
Cerebrum					
No abnormalities detected		11	5	1**	0**
Vacuolation of white matter	Total	39	45	49**	50**
	Trace	38	41	14	4
	Minimal	1	3	23**	16**
	Moderate	0	1	9**	14**
	Marked	0	0	3	16**
Cerebellum/pons/medulla					
No abnormalities detected		7	6	1*	0**
Vacuolation of white matter	Total	43	44	49*	49**
	Trace	37	33	18	11
	Minimal	6	9	18**	10
	Moderate	0	2	10**	10*
	Marked	0	0	3	18**
Spinal cord					
No abnormalities detected		13	14	5*	5**
Vacuolation of white matter	Total	37	36	45*	45**
	Trace	37	36	30	16
	Minimal	0	0	12**	8
	Moderate	0	0	2	13**
	Marked	0	0	1	8
Number examined		50	50	50	50

*: significantly different from control: p<0.05; **: significantly different from control: p<0.01 (Fisher's Exact Test)

Conclusion:

The principal target organ in mice after dietary administration of fluazinam at dose levels of 1000, 3000 and 7000 ppm for 97 and 104 weeks respectively was the liver. A statistically significant higher incidence of hepatocellular adenomas was observed in males receiving 3000 ppm (40%) compared to controls (16%) and to the historical control data of the

laboratory (max. 34 %). However, the incidence of hepatocellular adenomas in the highest dose group (28%) was within the incidence of adenomas in the historical controls.
Vacuolation of white matter was noted in the brain and cervical spinal cord in all dose groups.
To establish a NOAEL was not possible for this study.

B.6.5.3 Summary of long term toxicity/carcinogenicity studies

In the two long term toxicity/carcinogenicity studies in rats, treatment-related non-neoplastic effects were manifest at 100 ppm especially in the liver and testes. No treatment-related effects were seen on the spontaneous tumor profile at any dose level. Taking the two long term toxicity/carcinogenicity studies in rats together, an overall NOAEL for fluazinam can be obtained at 50 ppm, equivalent to 1.9 mg/kg bw/d for males and 2.4 mg/kg bw/d for females.
In two carcinogenicity studies in mice, liver cell tumours (adenomas and carcinomas) were observed in a greater number of male mice after dietary administration of 1000, 3000 and 7000 ppm fluazinam, reaching statistical significance for adenomas at dose levels of 1000 (33 %) and 3000 ppm (40 %) only. The historical control data for liver tumours carried out at Huntingdon Research Centre Ltd. in the years 1981 – 1983 and 1991 – 1993 showed incidences of adenomas in the range of 3.8 to 34 %. Thus the incidence of liver tumours at 1000 and 3000 ppm were within or slightly above the range of the historical control data. However, hepatocellular adenomas in the highest dose group of 7000 ppm reached an incidence of 28% and were within the range of the historical controls.
A statistically significant increase of vacuolation of white matter in the brain and cervical spinal cord was observed in both sexes at dose levels of 1000 ppm fluazinam and above. 10 ppm, equivalent to 1.12 mg/kg TG/d for males and 1.16 mg/kg TG/d for females, were considered to be the NOAEL in carcinogenicity studies in mice.

Table 6.5.3-1: Summarised results of long term toxicity studies with fluazinam

Study; Reference	Dose levels	NOAEL	Main effects/target organs
Sprague-Dawley rats 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.04, 0.38, 3.82 and 40 mg/kg bw males, 0, 0.05, 0.47, 4.87 and 53 mg/kg bw females)	10 ppm (0.38 mg/kg bw males, 0.47 mg/kg bw females)	-hematological and clinical chemical findings -higher liver and thyroid weights -histopathological changes in liver, pancreas, lungs and testes
Sprague-Dawley rats 104 weeks oral *Chambers P. R. et al; 1993*	0, 25, 50 and 100 ppm/diet (equivalent to 0, 1.0, 1.9 and 3.9 mg/kg bw males, 0, 1.2, 2.4 and 4.9 mg/kg bw females)	50 ppm (1.9 mg/kg bw males, 2.4 mg/kg bw females)	-higher liver, testes and epididymides weights -histopathological changes in liver, pancreas, lungs and testes
CD-1 mice 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.12, 1.12, 10.72 and 107 mg/kg bw males, 0, 0.11, 1.16, 11.72 and 117 mg/kg bw females)	10 ppm (1.12 mg/kg bw males, 1.16 mg/kg bw females)	-higher liver weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord
CD-1 mice 104 weeks oral *Chambers P. R. et al; 1998*	0, 1000, 3000 and 7000 ppm/diet (equivalent to 0, 126, 377 and 964 mg/kg bw males, 0, 162, 453 and 1185 mg/kg bw females)	Cannot be determined	-higher liver, brain and adrenal weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord

B.6.6 Reproductive toxicity (Annex IIA 5.6)

B.6.6.1 Single and multi-generation studies in rats

B-1216: Effects upon reproductive performance of rats treated continuously throughout two successive generations

Reference.: *Tesh J. M. et al; 1987;* Report No. 87/ISK068/097

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.

The study is in compliance with GLP.

Material and method:

Groups of 24 male and 24 female rats (strain: CD (Sprague-Dawley); source: Charles River, U.K. Limited, Margate, Kent), approximately 6 weeks old at beginning of treatment, received diets containing 0, 20, 100 or 500 ppm fluazinam (batch 8412-20, purity 95.3 %). F_0 animals were treated for 11 weeks prior to mating, throughout mating, gestation and lactation period until terminal sacrifice. Duration of mating period was 20 days on the basis of one male to one female. Litter size was standardised to 4 pups/sex/litter on day 4 post partum. Following weaning, the F_1 generation was selected, 24 animals/sex/group, and received treatment for 11 weeks before pairing to produce the F_2 generation. The study was terminated after weaning of the F_2 offspring. F_1 pups not selected to generate the second generation and all F_2 pups were sacrificed after weaning and were examined externally and internally. F_0 adults

were sacrificed shortly after the last F_1 pups were weaned and F_1 adults shortly after the last F_2 pups were weaned.

The average achieved intakes of fluazinam for the F_0 generation were equivalent to 0, 1, 5 and 26 mg/kg bw in males and 0, 1.4, 6.7 and 34 mg/kg bw in females. For the F_1 generation the average achieved intakes were 0, 1, 6 and 30 mg/kg bw in males and 0, 1.5, 7.5 and 40 mg/kg bw in females (lowest value of the range).

Diets were prepared weekly; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Food consumption was measured weekly. Body weights were recorded weekly through mating and on gestation days 0, 6, 13 and 20 and lactation days 1, 4, 7, 14 and 21 in females. The estrus cycle, mating performance and fertility were recorded. Offspring was observed for clinical signs and mortality and body weights were recorded on days 1, 4, 7, 11, 14 and 21 after birth. Physical development was assessed on a litter basis based on pinna unfolding, hair growth, tooth eruption and eye opening.

Mating performance: vaginal smears were taken each morning following pairing and examined for the presence of spermatozoa. The day on which evidence of mating was found was designated day 0 of gestation.

Parameters were calculated as follows:

Percentage mating: animals mating/animals paired X 100

Conception rate: animals achieving a pregnancy/animals mating X 100

Fertility index: number of live litters born/number of pregnant females X 100

Gestation index: animals achieving a pregnancy/animals paired X 100

Gestation length: taken as the time between the day of successful mating and the day on which pups were first seen.

Specified organs from all F_0 and F_1 parental animals (liver, ovaries, prostate with seminal vesicles, testes with epididymides and uterus) were weighed. Histopathological examinations were performed on these organs and on vagina, pituitary (animals of suspect fertility) and on all abnormalities from control and high dose animals of the F_0 and F_1 generation.

Examination was extended to the livers of males from the lowest and intermediate dietary concentration groups. Mammary tissue from any female which showed total litter loss was also examined microscopically.

Findings:

For both generations and both sexes, mean food consumption of treated animals of the low and intermediate groups was not different compared to control groups. F_0 females and both sexes of the F_1 generation of the high dose group showed a slight reduction in food intake during maturation. Body weight and body weight gain of F_0 females of the 500 ppm group was reduced during maturation and early gestation periods. Throughout the lactation period, body weight was similar to that of controls. Body weight and body weight gain was significantly reduced for females of the F_1 generation receiving 500 ppm during the maturation and gestation periods. Weight gain of females of the intermediate group (100

ppm) was slightly reduced during the gestation period. Reduced body weight was also recorded in F_1 females of the 500 ppm group at the lactation period (table 6.6.1-1).

Table 6.6.1-1 Mean group body weights of parental animals

F_0 mean parental body weight (g), premating period								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Week	Males	Females	Males	Females	Males	Females	Males	Females
0	189	146	187	147	188	147	187	146
11	535	298	539	291	537	290	530	270***
F_1 mean parental body weight (g), premating period								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Week	Males	Females	Males	Females	Males	Females	Males	Females
0	72	67	73	65	72	67	70	64
11	522	286	516	286	515	284	476	251***
F_0 mean maternal body weight (g) during gestation								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Day 0	289		294		292		272	
Day 6	321		328		322		298*	
Day 13	350		356		349		325*	
Day 20	416		423		416		388	
F_1 mean maternal body weight (g) during gestation								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Day 0	291		288		289		257***	
Day 6	316		315		313		278	
Day 13	347		346		339*		305	
Day 20	414		414		397*		359**	
F_0 mean maternal body weight (g) during lactation								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Day 1	324		324		315		301	
Day 21	343		347		340		330	
F_1 mean maternal body weight (g) during lactation								
	0 ppm		**20 ppm**		**100 ppm**		**500 ppm**	
Day 1	314		313		315		281**	
Day 21	338		334		329		302	

*: significantly different from control at p<0.05; **: significantly different from control at p<0.01; ***: significantly different from control at p<0.001 (t-test)

Mating performance, pregnancy rate and gestation index of the F_0 generation were not adversely affected by treatment at any dose level. Gestation length was slightly increased in the high dose group. Implantation sites and mean litter sizes were within the laboratory

background control ranges. In the F_1 generation, conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups. Numbers of implantation sites and mean litter sizes to day 4 post partum were slightly reduced for F_1 animals of the high dose group and marginally lower in the intermediate group (100 ppm). In both generations, survival and lactation indices and sex ratios were unaffected by treatment. Birthweight of F_1 was similar in all groups but body weight gain during lactation period was reduced in the 500 ppm group. At birth, bodyweights of F_2 pups of the 500 ppm and 100 ppm groups were slightly increased compared to controls, whereas bodyweight gain of offspring to weaning was reduced at 500 ppm.

The rate of physical development (pinna unfolding, hair growth, tooth eruption and eye opening) of F_1 offspring was similar in all dose groups, although onset and completion of eye opening was slightly earlier at 500 ppm. In the F_2 offspring, physical development was slightly more advanced at 500 ppm compared to controls (tables 6.6.1-2 and 6.6.1-3).

Table 6.6.1-2: Mating performance, fertility and litter data (F0 generation, mean group values)

	0 ppm	20 ppm	100 ppm	500 ppm
Gestational length (days)	22.5	22.5	22.5	23
Conception rate (%)	96	100	96	100
Fertility index (%)	96	100	96	100
Implantation sites	15.0	15.5	16.0	14.3
	14.8	14.5	14.2	14.5
Litter size total day 1	13.2	14.2	14.4	12.4
Litter size live day 1	13.0		13.6	11.9
Litter size live day 4	7.8	13.8	7.8	7.4
	88	7.9	91	88
Litter size live day 21		92		
	94		95	87
Post implantation survival index (%)		98		
Viability index (%)	100		100	99
Lactation index (%) day 7 p.p.		100		
	99		99	97
Lactation index (%) day 21 p.p.		100		
mean pup weight (g) day 1 p.p.	6.3	6.1	6.2	6.1
mean pup weight (g) day 4 p.p. (before cull)	9.0	8.3	8.3	8.5
mean pup weight (g) day 21 p.p. (postcull)	53.5	51.7	52.0	48.4***
Pinna unfolding, completion (day p.p.)	3.3	3.4	3.5	3.1
Hair growth, completion (day p.p.)	3.0	3.3	3.3	3.3
Tooth eruption, completion (day p.p.)	10.5	11.2	10.8	10.7
Eye opening, completion (day p.p.)	14.7	14.7	14.5	13.8**

: significantly different from control at p<0.01; *: significantly different from control at p<0.001: (Student's-test)

Table 6.6.1-3: Mating performance, fertility and litter data (F1 generation, mean group values)

	0 ppm	20 ppm	100 ppm	500 ppm
Gestational length (days)	22.5	22.5	23	23
Conception rate (%)	91	91	87	75
Fertility index (%)	87	88	83	75
Implantation sites	15.3	15.1	13.1	12.2*
Litter size total day 1	14.0	14.3	12.0	10.8**
Litter size live day 1	13.4	14.2	11.9	11.2
Litter size live day 4	12.4		11.3	9.8*
	7.4	12.8	7.3	6.8
Litter size live day 21	89	7.7	91	88
		93		
Post implantation survival index (%)	88		85	87
Viability index (%)		90		
Lactation index (%) day 7 p.p.	92		97	98
		99		
Lactation index (%) day 21 p.p.	90		96	97
		98		
mean pup weight (g) day 1 p.p.	5.8	5.7	6.2	6.2
mean pup weight (g) day 4 p.p. (before cull)	7.7	7.4	8.6	8.1
mean pup weight (g) day 21 p.p. (postcull)	50.8	48.3	51.4	45.5**
Pinna unfolding, completion (day p.p.)	3.9	4.1	3.4	3.2**
Hair growth, completion (day p.p.)	3.8	3.8	3.3	3.2**
Tooth eruption, completion (day p.p.)	10.9	11.0	10.9	10.2
Eye opening, completion (day p.p.)	14.8	15.0	14.7	14.1**

*: significantly different from control at p<0.05; ** significantly different from control at p<0.01;
***: significantly different from control at p<0.001 (Student's-test)

Pathology: Necropsy of adults and offspring in both generations revealed no adverse treatment related effects. Increased absolute liver weights, although not statistically significant, were seen in F_0 females of all treated groups and in F_0 males receiving 500 ppm. Relative liver weights were significantly increased in both sexes of the highest dose group and also in females of the intermediate and low dose group, but a clear dose response was not observed. A slight reduction in the absolute weight of ovaries of F_0 females receiving 500 ppm was also observed, related to body weight, however, there was no difference to controls. In F_1 animals receiving 500 ppm, significantly reduced bodyweight at necropsy was associated with slightly reduced absolute weights of epididymides and statistically significant reduced absolute weights of ovaries and liver (females only). When organ weights were related to bodyweight, however, the only statistically significant finding was an increase in liver weight in males receiving 500 ppm (table 6.6.1-4).

Table 6.6.1-4: Absolute (g)/relative (%) organ weights of F0 and F1 parental animals (mean group values)

	0ppm	20 ppm	100 ppm	500 ppm
F0 males, liver	22.3/3.67	22.1/3.61	22.2/3.71	23.3/3.95**
F0 females, liver	13.5/4.22	14.3/4.44*	14.0/4.43*	14.0/4.73**
F0 females, ovaries	0.104/0.0325	0.105/0.0326	0.124/0.0388	0.091*/0.0308
F1 males, liver	22.5/3.61	21.3/3.51	23.1/3.78	21.7/3.91**
F1 females, liver	12.8/3.95	13.3/4.12	13.0/4.0	11.7*/4.08
F1 females, ovaries	0.102/0.0318	0.106/0.0328	0.099/0.0307	0.083**/0.0290
F1 males, epididymides	1.367/0.2218	1.269/0.2100	1.333/0.2197	1.261/0.2288

*: significantly different from control at p<0.05; **: significantly different from control at p<0.01 (Dunnett's test)

Histopathological examination of the reproductive organs of controls and high dose group males and females of F_0 and F_1 adults revealed no changes considered to be of toxicological importance. Livers of F_0 and F_1 males of the 500 ppm group and also of F_1 males of the 100 ppm group showed an statistically significant increase of periacinar hepatocytic fatty changes. Livers of F_1 females of the 500 ppm group showed a statistically significant decrease of centriacinar fatty changes.

Conclusion:

Under the conditions of this study, rats fed a diet containing fluazinam in the highest concentration of 500 ppm over two generations showed statistically significant reductions in body weight and body weight gain of F_0 and F_1 parental females during maturation and gestation and of F_1 and F_2 offspring during lactation. Reduced food intake was recorded for F_0 females and F_1 males and females during maturation. In the F_1 generation, conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups. Numbers of implantation sites and mean litter sizes to day 4 post partum were slightly reduced for F_1 animals of the high dose group and marginally lower in the intermediate group (100 ppm). Relative liver weights were significantly increased in both sexes of the highest dose group and also in females of the intermediate and low dose group of the F_0 generation but there was no clear dose response observed. High dose males of the F_1 generation showed also an increase of relative liver weight. Histopathologically, an statistically significant increase of periacinar hepatocytic fatty changes were detected in high dose males of F_0 and F_1 animals and also in F_1 males of the 100 ppm group.

The NOAEL for both systemic toxicity and reproductive parameters was considered to be 20 ppm, equivalent to approximately 1 mg/kg bw/d for males and 1.4 mg/kg bw/d for females.

B.6.6.2 Developmental toxicity studies

Teratology study in the rabbit:

Reference.: *Tesh J. M. et al; 1985;* Report No. 85/ISK049/045

Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.

The study is in compliance with GLP.

Material and method:

Groups of 20 mated female New Zealand White rabbits (source: C. and J. Morton (Stansted) Ltd., Parsonage Farm, Essex, England), received oral doses (gavage) containing 0.3, 1 and 3 mg/kg bw fluazinam (batch Lot 8303-2, purity 98.5 %) from day 6 to 19 of gestation. 24 animals served as controls, receiving the vehicle 1 % w/v aqueous methylcellulose mucilage by intubation. Diets were prepared daily; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Animals were checked daily for mortalities or signs of reaction. Food consumption was recorded for each animal during the following phases of the study: days 1 - 5, days 6 – 12, days 13 – 19, days 20 – 23 and days 24 - 28 post coitum, body weights were recorded daily from day 0 until 28 post coitum. On day 29 post coitum, females were killed and the fetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. Liver and lungs were retained from all animals. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. All fetuses were dissected and examined internally.

Findings:

The general condition of the treated females was similar to that of the controls throughout the study. 4 animals of the control group and one in each of the 1 and 3 mg/kg bw/d group died during the study due to a Pasteurella infection. Mean food consumption of animals treated with 3 mg/kg bw/d fluazinam was slightly, but not statistically significant, reduced during the latter half of the dosing period. At 0.3 and 1 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.

Necropsy findings: There were no macroscopic changes in does at terminal necropsy which were considered treatment-related.

Reproduction data: One female in each of the 0.3 and 3 mg/kg bw/d dose groups aborted following weight loss. Necropsy revealed evidence of respiratory tract disorder. Number of implantations and viable young, the extent of pre- and post implantation loss and mean fetal and placental weights were unaffected by treatment.

Skeletal examination of fetuses revealed a reduction in the degree of ossification of long bones in the high dose group, which marginally exceeded the background control range. A slight dosage-related reduction in the degree of ossification of the phalangeal and metacarpal bones was also observed (tables 6.6.2-1 and 6.6.2-2).

Table 6.6.2-1: Reproduction data for does treated with fluazinam (mean group values)

Dose (mg/kg/bw/d)	0	0.3	1	3
No. of mated females	24	20	20	20
Not pregnant	1	3	3	4
Mortality	4	0	1	1
Abortion	0	1	0	1
Total litter loss	0	0	1	0
Pregnant to term with live young	18	16	15	14

Table 6.6.2-2: Percentage of mean fetal observations at skeletal examination (number of litters)

Dose (mg/kg/bw/d)	0	0.3	1	3	Historical control data (range)
Incomplete ossification of long bones	37.2 (15)	43.1 (14)	41.7 (14)	68.9 (13)	1.9 - 63.2
Incomplete ossification of phalangeal and/or metacarpal bones	8.3 (6)	15.7 (8)	17.6 (5)	20.8 (8)	1.9 – 53.2

Conclusion:

Under the conditions of this study, a NOAEL for maternal toxicity of 1 mg/kg bw/d can be obtained, based on reduced food intake in the high dose group. The NOAEL for fetal toxicity can be established at 1 mg/kg bw/d also, based on incomplete ossification in the high dose group.

There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d).

Teratology study in the rabbit:

Reference.: *Tesh J. M. et al; 1988;* Report No. 86/ISK069/324

The study was conducted according to current requirements of the U.S. E.P.A. Guideline No. 83-3 and Japanese M.A.F.F. and is in compliance with GLP. The study is considered acceptable.

Material and method:

4 groups of 16 to 17 mated female New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England), approximately 21 to 40 weeks old at commencement of the study, received oral doses (gavage) containing 2, 4, 7 and 12 mg/kg bw fluazinam (batch Lot 8412-20, purity 95.3 %) from day 6 to 19 of gestation. 18 animals served as controls, receiving the vehicle 1 % w/v aqueous methylcellulose mucilage by intubation. Diets were prepared daily; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Animals were checked daily for mortalities or signs of reaction. Food consumption was recorded for each animal during the following phases of the study: days 1 - 5, days 6 – 12, days 13 – 19, days 20 – 23 and days 24 - 28 post coitum.

Body weights were recorded each day prior to dosing and mean values were calculated on days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. On day 29 post coitum, females were killed and the fetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. Liver and lungs were retained from all animals. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. All fetuses were dissected and examined internally. Placentae were weighed and examined for external abnormalities.

Findings:

The general condition of the treated females was similar to that of the controls throughout the study. Mean food consumption of animals treated with 7 and 12 mg/kg bw/d fluazinam was reduced throughout the dosing period, statistically significant during the second half of the dosing period. In the 4 mg/kg bw/d group, food consumption was reduced during the second half of the dosing period too, but statistical significance was not reached. At 2 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.

Absolute maternal body weights in animals dosed at concentrations of 2, 4 and 7 mg/kg/day were comparable to controls. Mean body weights in 12 mg/kg/day dosed animals were lower than concurrent controls from day 10 through Day 20 of gestation, reaching statistical significance on day 20. The body weights were increased during the postdosing period and the animals had recovered approximately 50% of their body weight losses by termination (table 6.6.2-3).

Table 6.6.2-3: Mean maternal body weight (kg) during gestation

Dose	Day of Gestation										
Mg/kg	0	6	8	10	12	14	16	18	20	24	28
0	3.90	4.00	4.03	4.08	4.13	4.18	4.26	4.29	4.33	4.37	4.40
2	4.05	4.09	4.14	4.16	4.18	4.22	4.28	4.27	4.26	4.34	4.40
4	4.03	4.14	4.17	4.21	4.24	4.27	4.26	4.29	4.33	4.34	4.43
7	3.99	4.10	4.14	4.16	4.16	4.21	4.21	4.23	4.25	4.34	4.41
12	3.92	3.99	4.03	4.07	4.06	4.08	4.05	4.07	4.07*	4.23	4.25

*: significantly different from control at p<0.05

Necropsy findings: Macroscopic examination showed respiratory tract infection and areas of discolouration or pallor of livers in animals of the 4, 7 and 12 mg/kg bw/d groups. Microscopic changes included hepatocytic hypertrophy, increased apoptosis, necrosis/degeneration of single hepatocytes, increased brown pigment within the hepatocytes, focal hepatocytic necrosis, bile plugs and an increase in the number of binucleate hepatocytes. Statistical significance was reached in the 7 and 12 mg/kg bw/d

groups (table 6.6.2-4).

Table 6.6.2-4: Microscopic Findings in the liver (16 animals/dose group) of does

	Dose (mg/kg bw/day)				
Finding	**0**	**2**	**4**	**7**	**12**
Increased apoptosis	0	0	0	2	2
Necrosis of occasional single hepatocyte	0	0	0	2	4
Hepatocytes containing increased brown pigment	0	0	0	3	2
Foci of hepatocytic necrosis	0	0	0	0	2
Occasional bile plugs within distended canaliculi	0	0	0	0	1
Centriacinar hypertrophy, slight	0	0	2	2	0
Panacinar hypertrophy, slight	0	0	1	3	2
Moderate	0	0	0	2	5
Marked	0	0	0	0	2

Reproduction data: Two females in each of the 4 and 7 mg/kg bw/d dose groups and one in the 12 mg/kg bw/d group aborted during the study. Total resorption was observed in one animal of the 7 mg/kg bw/d group and in 5 animals of the 12 mg/kg bw/d group (table 5.6.2-5).

Table 6.6.2-5: Reproduction data for female rabbits treated with fluazinam (mean group values)

Dose (mg/kg/bw/d)	**0**	**2**	**4**	**7**	**12**
No. of mated females	18	16	17	17	16
Not pregnant	1	2	3	1	1
Mortality	2	1	2	3	2
Abortion	0	0	2	2	1
Total litter loss	0	0	0	1	5
Pregnant to term with live young	15	13	10	10	7

Preimplantation loss was elevated in all treated groups in comparison to the concurrent controls, but all values fell within the recorded background control range of the laboratory (4.7 – 35.7 % in 92 studies). Postimplantation loss was increased at 4 mg/kg/day compared to concurrent controls, however, no increase was observed at the 7mg/kg/day dose level. A significant postimplantation loss was noted for the 12 mg/kg bw/d group. Fetal and placental weights were similar in all groups to concurrent control responses. There was a complete litter loss for 5 high-dose females and for one of the 7 mg/kg/day dose groups. No complete litter loss could be observed in controls and 2 and 4 mg/kg/day dose groups (table 6.6.2-6).

Table 6.6.2-6: Group mean litter data for female rabbits treated with fluazinam

Dose (mg/kg/bw/d)	0	2	4	7	12	Recorded ranges in 92 studies
Corpora lutea count	11.3	11.3	10.6	10.6	10.6	9.3 – 13.5
Implantations	9.9	8.2	8.5	7.7	7.9	6.5 – 11.0
Viable young	9.1	7.3	6.3	7.2	6.3	5.5 – 9.8
Resorptions total **early** **late**	0.9 0.7 0.2	0.9 0.5 0.5	2.2 0.7 1.5	0.5 0.4 0.1	1.6 0.7 0.9	0.1 – 1.7 0.0 – 1.1 0.0 – 1.4
Preimplantation loss (%)	12.4	27.2	19.8	27.4	25.7	4.7 – 35.7
Postimplantation loss (%)	8.7	11.2	25.9	6.5	20.0	1.0 – 20.5
Fetal weight (g)	40.4	43.2	44.3	42.5	41.4	36.1 – 46.9
Placental weight (g)	5.4	6.5	6.3	6.0	5.9	5.0 – 7.2

There were several abnormalities noted in fetuses during the external and visceral examination of all treatment groups, but mainly in the high-dose group. Several findings in the high-dose group were found only in a single litter or were within the historical control range. However, for placental anomalies (not nearer specified), the incidence was above the historical control range for the laboratory and appears to be due to treatment.

The incidence of several skeletal abnormalities was clearly increased in the high-dose group over both the study control values and the historical control range for the laboratory. Effects that may be treatment related include kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones (table 6.6.2-7).

Table 6.6.2-7: Percentage of fetal observations at skeletal examination (number of litters)

	Dose (mg/kg/day)					
Parameter	0	2	4	7	12	Historical control data (range)
Fetuses (litters)	136(15)	95(13)	63(10)	72(10)	44(7)+	86 - 92 studies 8407 –9385 fetuses
Placental anomalies	0.7(1)	3.2(3)	0.0	0.0	18.2(3)+	0.0 – 16.3
Head: additional sutures, parietal bones	0.7(1)	0.0	3.2(2)	2.8(2)	6.8(3)+	0.0 – 3.3
Incomplete ossification of sternebrae	0.0	0.0	0.0	0.0	2.3(1)+	0.0 – 1.1
Two or more sternebrae fused	2.2(2)	1.1(1)	1.6(1)	1.4(1)	9.1(2)+	0.0 – 5.3
Tail tip kinked	0.0	0.0	1.6(1)	0.0	4.5(2)+	0.0 – 2.6

+: Value above historical control high value

Conclusion:

Oral administration of fluazinam to pregnant rabbits during the period of organogenesis was associated with reduced maternal weight gain and food intake in the highest dose group of 12 mg/kg bw/d. Macroscopic and microscopic lung and liver changes were observed at a dose level of 4 mg/kg bw/d and above. So the maternal NOAEL can be considered at 2 mg/kg/day. Increased incidences of fetal abnormalities (placental abnormalities, some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were seen at the top dose. At all dose levels, increased incidences of preimplantation losses were observed, however, the values fell within the recorded background control range of the laboratory. Based on increased abortion and postimplantation loss from 4 mg/kg bw/d upwards, the NOAEL for developmental effects can be set at 2 mg/kg bw/d.

Teratology study in the rat:

Reference.: *Willoughby C. R. et al; 1984;* Report No. 84/ISK047/606 and amended Final Report No. 91/ISK047/0820

The study was conducted according to U.S. E.P.A. Guideline No. 83-3 and is in compliance with GLP. The study is considered acceptable.

Material and method:

3 groups of 20 mated female rats (strain: CD (Sprague-Dawley); source: Charles River, U.K. Limited, Margate, Kent), approximately 9 to 11 weeks old at commencement of the study, received oral doses (gavage) containing 10, 50 and 250 mg/kg bw fluazinam (batch Lot 8303-2, purity 98.5 %) from day 6 to 15 of gestation. 20 animals served as controls, receiving the vehicle (corn oil) by intubation. Diets were prepared daily; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Animals were checked daily for mortalities or signs of reaction. Food consumption was recorded for each animal during the following phases of the study: days 0 - 2, days 3 – 5, days 6 – 8, days 9 – 11, 12 – 15, 16 - 17 and days 18 - 19 post coitum. Body weights were recorded on days 0, 3, 6 to 16, 18 and 20 of gestation. On day 20 post coitum, females were killed and the fetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. Fetuses were dissected and examined internally. Placentae were weighed and examined for external abnormalities.

Findings:

14 animals in the high dose group (250 mg/kg bw/d fluazinam) showed urogenital staining during the dosing phase. In other respects, the general condition of the treated females was similar to that of the controls throughout the study. Mean food consumption of animals

treated with 250 mg/kg bw/d fluazinam was reduced statistically significant during the early dosing period. In the 50 mg/kg bw/d group, food consumption was reduced during the early part of the dosing period too, but statistical significance was not reached. At 10 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.

Animals dosed at concentrations of 250 mg/kg/day showed weight loss between days 6 and 8, followed by a slight reduced rate of weight gain between days 9 and 11 post coitum comparable to controls. Their rate of weight gain became slight superior to that of controls, although the overall weight gain from day 6 to 15 and to day 20 remained significantly reduced. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. At 10 mg/kg bw fluazinam, weight gain was unaffected (table 6.6.2-8).

Table 6.6.2-8: Mean maternal body weight gains (g) during gestation

Dose	Days post coitum			
mg/kg	0 – 6	6 – 15	16 - 20	6 – 20
0	34	51	52	116
10	34	50	54	116
50	33	46	56	112
250	35	30**	58	98**

**: significantly different from control at p<0.01 (Dunnetts t-test)

Necropsy findings: Macroscopic examination of dams on day 20 of gestation revealed no changes attributable to treatment.

Reproduction data: Numbers of implantations, live young and the extent of preimplantation loss were unaffected by treatment with fluazinam. Postimplantation loss was increased in the 250 mg/kg/day group compared to concurrent controls, however, not statistically significant and within the range of the historical controls of the laboratory. Fetal and placental weights were significantly reduced in the high dose group. In the 50 mg/kg bw/d group, fetal and placental weights were clearly reduced compared to controls. 10 mg/kg/day dose groups were unaffected by treatment with fluazinam (table 6.6.2-9).

Table 6.6.2-9: Group mean litter data for female rats treated with fluazinam

Dose (mg/kg/bw/d)	0	10	50	250	Ranges in 63 current studies	Ranges in 80 studies since 1982
Corpora lutea count	16.3	15.4	16.4	16.8	14.3 – 17.6	14.0 – 18.3
Implantations	14.4	14.1	15.2	15.0	12.7 – 15.8	11.6 – 16.5
Viable young	13.8	13.5	14.3	13.4	11.1 – 14.8	10.9 – 15.9
Resorptions total **early** **late**	0.6 0.55 0.05	0.6 0.55 0.05	0.85 0.75 0.1	1.65 1.1 0.55	0.32 – 1.65 0.05 – 1.47 0.0 – 0.58	0.08 – 1.91 0.08 – 0.53 0.0 – 1.45
Preimplantation loss (%)	12.0	8.1	7.3	11.2	4.0 – 15.8	2.6 – 20.9
Postimplantation loss (%)	4.2	4.3	5.6	11.0	2.1 – 12.7	0.5 – 14.0
Fetal weight (g)	3.19	3.19	3.11	2.81***	3.16 – 3.55	3.51 – 4.04
Placental weight (g)	0.54	0.53	0.49	0.47**	0.43 – 0.53	0.45 – 0.62

: significantly different from control at p<0.01; *: significantly different from control at p<0.001 (t-test)

Abnormalities were noted in the litters of four high-dose animals and included facial/palatal cleft and/or diaphragmatic hernia.Three litters had just one fetus with one of the abnormalities and the remaining litter with up to 8 fetuses with an abnormality (table 6.6.2-10).

Table 6.6.2-10: Incidences of facial/palatal clefts and/or incomplete ossification of palatine bones and diaphragmatic hernia in fetuses of high dose animals (250 mg/kg bw/d)

		Number of fetuses with	
Animal number	Number of fetuses examined	Facial/palatal cleft	Diaphragmatic hernia
1	14	0	1
2	12	1	0
3	11	1+	0
4++	17	8*	6*

*: 2 fetuses showed both anomalies; +: small fetus with incomplete ossification of palatine bones;
++: large litter size, low mean fetal weight

The skeletal examination showed a reduction in the degree of ossification of cranial bones, sternebrae, caudal vertebrae, metacarpals/metatarsals and pubic bones in high-dose fetuses. An increased frequency of 14th rib was seen at 50 mg/kg/day and higher (table 6.6.2-10).

Table 6.6.2-11: Percentage of fetal observations at skeletal examination (number of litters)

	Dose (mg/kg/day)					
Parameter	0	10	50	250	Current control data (range)	Historical control data since 1982 (range)
Fetuses (litters)	134 (20)	130 (20)	139 (20)	129 (20)	4605 (54 studies)	6493 (74 studies)
Cleft palate	-	-	-	2.3 (1)	-	-
Diaphragmatic hernia	-	-	-	3.1 (2)	0.0 - 1.3	0.0 – 0.6
Incomplete ossification of cranial bones	22.9 (14)	24.3 (15)	29.9 (17)	54.3 (19)	7.1 – 47.8	2.0 – 6.9
Incomplete ossification of sternebrae	20.7 (12)	17.9 (14)	29.9 (17)	32.6 (18)	1.1 – 23.3	0.9 – 47.2
Incomplete ossification of caudal vertebrae	6.4 (5)	3.6 (5)	8.2 (7)	13 (11)	0.0 – 12.4	0.6 – 1.2
Incomplete ossification of metacarpals/metatarsals	7.1 (5)	6.4 (6)	4.8 (7)	10.1 (9)	0.0 – 5.8	0.0 – 4.5
Incomplete ossification of pubic bones	10.7 (7)	15.7 (12)	12.2 (9)	22.5 (14)	0.0 – 16.0	0.0 – 3.1

The visceral examination revealed cardiac septal defects in one fetus in each of the control, 50 and 250 mg/kg bw/d groups. One fetus in the high dose group had an abnormal aortic arch and a septal defect.

Conclusion:

Oral administration of fluazinam at the high dose level of 250 mg/kg bw/d to pregnant rats during the period of organogenesis was associated with reduced mean food consumption followed by a reduced rate of weight gain compared to controls. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. So the maternal NOAEL can be considered at 10 mg/kg/day. Fetal and placental weights were significantly reduced in the high dose group and there were indications of fetal immaturity. In the 50 mg/kg bw/d group, fetal and placental weights were reduced compared to controls. An increased incidence of gross morphological fetal abnormalities were recorded at the top dose and values were outside the range of the concurrent controls and the recorded background controls of the laboratory. It can be concluded that fluazinam was teratogenic after oral application. The NOAEL for developmental effects can be set at 10 mg/kg bw/d.

B.6.6.3 Summary of reproductive toxicity

In a two generation reproduction study, rats fed a diet containing fluazinam in the highest concentration of 500 ppm showed statistically significant reductions in body weight and body

weight gain and reduced food intake. Relative liver weights were significantly increased in both sexes of the highest dose group and also in females of the intermediate and low dose group of the F0 generation, but a clear dose response was not observed. High dose males of the F1 generation showed also an increase of relative liver weight. Histopathologically, an statistically significant increase of periacinar hepatocytic fatty changes were detected in high dose males of F0 and F1 animals and also in F1 males of the 100 ppm group. The NOAEL for systemic toxicity was considered to be 20 ppm, equivalent to approximately 1 mg/kg bw/d for males and 1.4 mg/kg bw/d for females.

Reproductive performance of F0 animals was unaffected by treatment. In the F1 generation, conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups. Numbers of implantation sites and mean litter sizes to day 4 post partum were slightly reduced for F1 animals of the high dose group and marginally lower in the intermediate group (100 ppm). The NOAEL for reproductive parameters was considered to be 20 ppm, equivalent to approximately 1 mg/kg bw/d for males and 1.4 mg/kg bw/d for females.

Two teratology studies in rabbits had been performed. In the first study, dose levels of 0.3, 1 and 3 mg/kg bw fluazinam from day 6 to 19 of gestation had been chosen. There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d). In the high dose group of 3 mg/kg bw fluazinam, reduced food intake and incomplete ossification were observed. Based on these results, the NOAEL for maternal toxicity and fetal toxicity was obtained at 1 mg/kg bw/d.

In the second study, oral administration of fluazinam to pregnant rabbits during the period of organogenesis was associated with reduced maternal weight gain and food intake in the highest dose group of 12 mg/kg bw/d. Macroscopic and microscopic lung and liver changes were observed at a dose level of 4 mg/kg bw/d and above. So the maternal NOAEL was considered at 2 mg/kg/day. Increased incidences of fetal abnormalities (placental abnormalities, some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were seen at the top dose. At all dose levels, increased incidences of preimplantation losses were observed, however, the values fell within the recorded background control range of the laboratory. Based on increased abortion and postimplantation loss from 4 mg/kg bw/d upwards, the NOAEL for developmental effects was set at 2 mg/kg bw/d.

Taking the two teratology studies in rabbits together, an overall NOAEL for maternal and fetal toxicity can be obtained at 2 mg/kg bw/d.

In a teratology study in rats, oral administration of fluazinam at the high dose level of 250 mg/kg bw/d to pregnant rats during the period of organogenesis was associated with reduced mean food consumption and weight loss, followed by a slight reduced rate of weight gain compared to controls. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. So the maternal NOAEL was considered at 10 mg/kg/day. Fetal and placental weights were significantly reduced in the high dose group and there were

indications of fetal immaturity. In the 50 mg/kg bw/d group, fetal and placental weights were reduced, but not significantly, compared to controls. An increased incidence of gross morphological fetal abnormalities were recorded at the top dose, values were outside the range of the concurrent controls and the recorded background controls of the laboratory. So the NOAEL for developmental effects was considered at 10 mg/kg bw/d. According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam should be classified to "category 3 of reproductive substances" and labelled with the risk phrase "R 63 – Possible risk of harm to the unborn child".

Table 6.6.3-1: Summarised results of reproductive toxicity/teratogenicity studies with fluazinam

Study Reference	Dose levels	NOAEL	Main effects/target organs
Two generation reproduction, rats *Tesh J. M. et al; 1987*	0, 20, 100 or 500 ppm, equivalent to 0, 1, 5 and 26 mg/kg bw/d males; 0, 1.4, 6.7 and 34 mg/kg bw/d females (lowest value of the range)	Parental and Reproductive NOAEL 20 ppm (1 mg/kg bw/d males, 1.4 mg/kg bw/d females)	Parental: body weight and body weight gain ↓; relative liver weight ↑ Offsprings: gestation length ↑; implantation sites and litter sizes ↓
Teratology in the rabbit *Tesh J. M. et al; 1985*	0, 0.3, 1 and 3 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 1 mg/kg bw/d Developmental NOAEL 1 mg/kg bw/d	Maternal: food consumption ↓ Developmental: ossification incomplete
Teratology in the rabbit *Tesh J. M. et al; 1988*	0, 2, 4, 7 and 12 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 2 mg/kg bw/d Developmental NOAEL 2 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓; histopathological liver changes Developmental: abortion ↑; postimplantation loss ↑
Teratology in the rat *Willoughby C. R. et al; 1985*	0, 10, 50 and 250 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 10 mg/kg bw/d Developmental NOAEL 10 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓ Developmental: fetal and placental weight ↓; ossification incomplete; gross morphological fetal abnormalities

B.6.7 Neurotoxicity/delayed neurotoxicity (Annex IIA 5.7)

An acute neurotoxicity screening study in rats with technical fluazinam (IKF-1216)

Reference: *Serrone D.M.; 1995;* Report No. 5603-93-0075-TX-003

No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable and is in compliance with GLP.

Material and method:

Groups of 10 male and 10 female rats (strain: Crl:CD BR VAF/Plus rats; source: Charles River Breeding Laboratories, Portage, Michigan), 43 to 46 days old at treatment, received single oral doses (gavage) of 50, 1000 and 2000 mg/kg bw fluazinam (Lot Nr. 1030/91, purity 96.8 %, dispersed in 1.5 % (w/v) methylcellulose). 10 males and 10 females received a single dose of the vehicle alone (methylcellulose) and acted as controls. All animals were observed daily for clinical signs or reaction to treatment. Body weights were recorded prior to initiation of test material administration, on the day of treatment and on days 7 and 14. Food

consumption was recorded on a weekly basis. All animals were subjected to a functional observational battery (FOB) one week prior to treatment, at the time of peak effect 5 to 7 hours postdosing and 7 and 14 days after treatment. Motor activity, forelimb and hindlimb grip strength and landing foot-spread were also quantitatively assessed at the same intervals.

The functional observational battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed when initially handling the animal. The third set of observations was performed in a test arena. The fourth set comprised handling/specific testing of the animal.

Animals were killed at the end of the observation period (after 14 days). Five animals per sex in each group were taken for neuropathologic evaluation, the remaining animals were subjected to a gross necropsy examination.

Findings:

Mortalities were not observed.

Body weights for both sexes were similar for all groups including controls.

Soft stools were observed in both sexes of the 1000 and 2000 mg/kg bw groups on the day of treatment.

Neurotoxicity screening: The functional observational data at 5 to 7 hours post dosing and 7 and 14 days after treatment showed no differences in FOB categories between treated and control groups.

6 hours after dosing, motor activity of females treated with 1000 and 2000 mg/kg bw fluazinam was statistically significantly lower compared to controls. There were no similar effects in males. No statistically significant differences in those parameters could be observed 7 and 14 days after dosing.

Postmortem examinations:

No test material-related findings were observed at gross necropsy examinations.

There were no histopathological findings in the sections of nervous tissue examined (brain, medulla oblongata, skeletal muscle, spinal cord, sciatic and tibial nerve, sural nerve, gasserian ganglion, cervical dorsal root ganglion, cervical dorsal root, cervical ventral root, lumbar dorsal root ganglion, lumbar dorsal root, lumbar ventral root) from high dosed males and females. Tissues of the 50 and 1000 mg/kg bw groups were therefore not examined.

Conclusion:

Oral administration of 1000 and 2000 mg/kg bw fluazinam produced soft stool in both sexes on the day of treatment and statistically significantly lower motor activity in females compared to controls. No pathological findings were observed at gross necropsy examination and no histopathological findings were seen in the sections of nervous tissues examined. The NOAEL for neurotoxicity can be considered at 2000 mg/kg bw, the NOAEL based on systemic toxicity at 50 mg/kg bw.

IKF-1216 neurotoxicity to rats by dietary administration for 13 weeks

Reference: *Hughes E. W.; 1998;* Report No. ISK 251/971800

The study was conducted according to EPA FIFRA 81-8 and is in compliance with GLP. The study is considered acceptable.

Material and method:

Groups of 10 male and 10 female rats (strain: Crl:CD BR rats; source: Charles River Breeding Laboratories, Manston Road, Margate, Kent, England), approximately 35 days old at treatment, received diets containing 0, 300 and 1000 ppm fluazinam (Lot Nr. 6109, purity 96.9 %). Average achieved intakes were 0, 20.7 and 69 mg/kg bw in males and 0, 23.4 and 81 mg/kg bw in females.

Concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis.

All animals were observed daily for clinical signs or reaction to treatment. Body weights were recorded on the day of allocation to groups, on the day of commencement of treatment and once a week thereafter. Body weights were also recorded on each occasion the functional observational battery (FOB) was performed. Food consumption was recorded on a twice weekly basis. All animals were subjected to a functional observational battery prior to treatment and during the 4th, 8th and 13th week of treatment. Motor activity of each animal was also quantitatively assessed at the same intervals.

The functional observational battery comprised 4 sets of observations. The first set of observations was performed while the animal was in its home cage. The second set of observations was performed when initially handling the animal. The third set of observations was performed in a test arena. The fourth set comprised handling/specific testing of the animal.

At the end of the 13 weeks of treatment, remaining animals were killed and the tissues fixed by whole body perfusion. Examination was confined to designated tissues of the nervous system which were subsequently examined microscopically (neuropathological examination was restricted to 5 males and 5 females from the control and high dose groups).

Findings:

There were no clinical signs of reaction to treatment and no mortalities. Body weight gains among females treated with 1000 ppm fluazinam were statistically significantly lower than controls. Among males at this dose level, weight gains were compareable to controls.

Neurotoxicity screening: During week 4, males of the 1000 ppm-group showed a lower level of arousal and there was a statistically significantly increase of males urinating in the arena compared to controls. Females at this dose level groomed while in the arena. During week 8, both males and females of the 1000 ppm-group groomed while in the arena. Slightly more males of the 300 ppm and 1000 ppm-groups urinated in the arena compared to controls. During week 13, males of the 1000 ppm-group showed a lower level of arousal and there was a slightly higher incidence of males for which assessment of gait was not possible. During week 4, rearing counts of males of the 1000 ppm-group were statistically significantly

lower compared to controls. Females at this dose level showed no effects. There were no differences in rearing counts between treated and control groups during weeks 8 and 13. There were no statistically significant differences in fore- and hindlimb grip strength or hindlimb splay of both sexes on any occasion of testing.

There was no difference in locomotor activity compared to controls during weeks 4 and 13. During week 8, males of both dose groups showed statistically significant lower locomotor activity after 30 minutes of activity.

Group mean rectal temperature was compareable among all groups including controls.

There were no histopathological findings in the sections of nervous tissue examined which were considered treatment related.

Conclusion:

There was no evidence of neurotoxicity during the course of the study nor was there any evidence of neuropathology after 13 weeks of treatment. Lower body weight gains were observed only in females of the 1000 ppm-group compared to controls. As there were no statistically significant differences in locomotor activity compared to controls during week 13, the change observed in males during week 8 was not considered to be treatment related. The NOAEL for neurotoxicity was established at 1000 ppm (69 mg/kg bw). The NOAEL for systemic toxicity was established at 300 ppm (21 mg/kg bw/d), based on statistically significantly lower body weight gains among females treated with 1000 ppm fluazinam.

B.6.7.1 Summary of Neurotoxicity

Single oral doses (gavage) of 1000 and 2000 mg/kg bw fluazinam produced statistically significantly lower motor activity in female rats compared to controls. No pathological findings were observed at gross necropsy examination and no histopathological findings were seen in the sections of nervous tissues examined. The NOAEL based on systemic toxicity was considered to be 50 mg/kg bw.

After 13 weeks of treatment with fluazinam in the diet, no evidence of neurotoxicity and neuropathology during the course of the study was observed. Reduced locomotor activity observed in males during week 8 of treatment compared to controls was not considered to be treatment related as there were no statistically significant differences during week 13. The NOAEL for neurotoxicity was established at 1000 ppm (69 mg/kg bw). The NOAEL for systemic toxicity was established at 300 ppm (21 mg/kg bw/d), based on statistically significantly lower body weight gains among females treated with 1000 ppm fluazinam.

B.6.8 Further toxicological studies (Annex IIA 5.8)

B.6.8.1 Toxicity studies on metabolites

Additional studies were performed with HYPA (G-450), chemical name 5-((3-chloro-5-(trifluoromethyl)-2-pyridyl)amino)- α,α,α- trifluoro-4,6-dinitro-o-cresol and MAPA (G-525), chemical name 2-(2-amino-3-chloro-α,α,α-trifluoro-6-nitro-*p*-toluidino)-3-chloro-5-(trifluoromethyl)pyridine, which were both found in liver, kidney, muscle, fat and eggs of

Laying hens but not in rat metabolism studies.

Acute oral toxicity to mice of G-450:

Reference: *Liggett, M. P.; 1988;* Report No. 881245D/ISK19/AC

Guideline: The study was conducted according to Japanese MAFF Test Guidelines, 59 NohSan No. 4200 (1985).

GLP: yes

Material and Methods:

Groups of 5 mice/sex (strain: CFLP; source: Interfauna UK Ltd., Huntingdon) weighing between 16 and 26 g (4 - 6 weeks old) received single oral doses of 160, 250, 400 and 640 mg/kg bw G-450, HYPA, (Lot No. 8805, purity 98.8%) suspended as a w/v formulation in aqueous methylcellulose by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All mice were necropsied and macroscopic organ changes reported.

Findings:

Clinical signs and mortality:

Signs of reaction to treatment at 160 mg/kg bw and above were limited to pilo-erection, hunched posture and pallor of the extremities in all mice from within one hour of dosing. Abnormal body carriage, waddling, lethargy, decreased respiratory rate, ptosis, ataxia and prostration were observed amongst mice treated at 250 mg/kg bw and above. Recovery of surviving mice was complete by day 2 and 3 (160 mg/kg bw), day 3 and 4 (250 mg/kg bw) or day 4 (400 mg/kg bw). Treatment-related deaths were observed amongst females dosed at 250 mg/kg bw and above and amongst both sexes treated at 400 mg/kg bw and above. Deaths occurred from within five hours of dosing until day 4.

Pathology:

No macroscopic organ changes were noted in mice that died or in any of the surviving animals at necropsy.

Conclusion:

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of HYPA in mice of both sexes observed for a period of 15 days was 331 mg/kg bw.

Acute oral toxicity to mice of G-525:

Reference: *Liggett, M. P.; 1988;* Report No. 881248D/ISK19/AC

Guideline: The study was conducted according to Japanese MAFF Test Guidelines, 59 NohSan No. 4200 (1985).

GLP: yes

Material and Methods:

Groups of 5 mice/sex (strain: CFLP; source: Interfauna UK Ltd., Huntingdon) weighing between 21 and 25 g (4 - 6 weeks old) received a single oral dose of 5000 mg/kg bw G-525,

MAPA (Lot No. 8805, purity 99.8 %), suspended as a w/v formulation in aqueous methylcellulose by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All mice were necropsied and macroscopic organ changes reported.

Findings:

Clinical signs and mortality:

Signs of reaction to treatment was limited to pilo-erection shortly after dosing and throughout the remainder of day 1. Recovery was complete by day 2. Treatment-related deaths were not observed.

Pathology:

No macroscopic organ changes were noted in mice at necropsy.

Conclusion:

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of MAPA in mice of both sexes observed for a period of 15 days was > 5000 mg/kg bw.

Bacterial reverse mutation test of G-450 (HYPA):

Reference.: *Ohtsuka M.; 1989;* Report No. T-1676E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of G-450, HYPA, (Lot No. 8805, purity 98.8%) in bacterial systems. The *S. typhimurium* (TA100, TA1535, TA98 and TA1537) and *E. coli* (WP2 uvr A) tests were performed with 125, 250, 500, 1000, 2000 and 4000 µg/plate in a DMSO solution without metabolic (S-9) activation and with 313, 625, 1250, 2500 and 5000 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). Dose levels were established on the basis of a preliminary range finding test.

Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.

Evaluation criteria:

The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose response.

Findings:

The results of the tests showed a dose-dependent increase in the number of revertant

colonies of slightly more than twice that of the solvent control for S. typhimurium TA98 in the absence of S-9 mix. The other bacteria tester strains exposed to G-450 (HYPA) showed no increase in the number of revertant colonies at any dose level, either with or without metabolic activation. AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).

Conclusion:

The results of the test indicate that G-450 (HYPA) showed only slight reverse mutagenicity against S. typhimurium TA98 without S-9 mix at the concentrations tested. The other bacteria tester strains showed no increase in the number of revertant colonies at any dose level.

HYPA Bacterial reverse mutation test:

Reference: *May K.; 2002;* Report No.ISK 270/024536;

Guideline: The study was conducted according to OECD Guideline 471 (1997); EEC Directive 2000/32/EC Annex 4D-B.13/14; U.S. EPA Health Effects Guidelines, OPPTS 870.5100, EPA 712-C-98-247; Japanese MAFF, NohSan No. 8147 (2000) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

HYPA (batch 0006, purity 99.7%) was tested in the Ames test. Histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA1535, TA1537, TA98 and TA100) and a tryptophan dependent mutant of *Escherichia coli*, strain WP2*uvr*A were exposed to HYPA diluted in dimethylsulfoxide (DMSO), which was also used as a negative control. Positive controls were, in the absence of S-9 mix, *sodium azide* (0.5 µg/plate for strains TA1535 and TA100), *9-aminoacridine* (50 µg/plate for strain TA1537), *2-nitrofluorene* (1µg/plate for strain TA98) and *2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2)* (0.05 µg/plate for strain WP2uvrA). In the presence of S-9 mix, *2-aminoanthracene* (2 µg/plate for strain TA1535 and 10 µg/plate for strain WP2uvrA) and *benzopyrene* (5 µg/plate for strains TA1537, TA98 and TA100) were used.

Two independent mutation tests were performed in the presence and absence of liver preparations from Aroclor 1254-induced rats (S-9 mix). A third test was conducted in three strains in the absence of S-9 mix. The first (range-finding) test was a standard plate incorporation assay, the second and third involved a pre-incubation stage.

First test: HYPA was added to cultures of the five tester strains at seven concentrations separated by *ca* half-$\log_{10}$ intervals. Dose concentrations were 5, 15, 50, 150, 500, 1500 and 5000 µg/plate. The highest concentration is the standard limit dose recommended in the regulatory guidelines this assay follows. An aliquot of 0.1 ml of a bacterial culture 10-hours after cultivation and 0.5 ml S-9 mix or 0.5 ml 0.1 M sodium phosphate buffer (pH 7.4) were placed in glass bottles. An aliquot of 100 µl of the test solution was added, followed

immediately by 2 ml of molten agar containing 0.05mM histidine/biotin/tryptophan. The mixture was shaken and overlaid onto petridishes containing 25 ml minimal agar. Three petridishes were used for each dose level. Plates were also prepared without the addition of bacteria in order to assess the sterility of the test substance, S-9 mix and phosphate buffer. All plates were incubated at 37° C for 72 hours. After this period the appearance of the background bacterial lawn was examined and revertant colonies counted using a Domino automated colony counter.

Second test: As a clear negative response was obtained in the first test, a variation to the test procedure was used for the second. The variation used was the pre-incubation assay in which the bottles which contained mixtures of bacteria, buffer or S-9 mix and test solution, were incubated at 37° C for 30 minutes with shaking before the addition of the agar overlay. 5000 µg/plate was again chosen as the top concentration (except strain TA1537: 1500 µg/plate was selected for testing in the absence of S-9 mix), with a total of five dose levels (50, 150, 500, 1500 and 5000 µg/plate).

Third test: As insufficient non-toxic dose levels were obtained in strains TA1535, TA1537, TA98 following pre-incubation in the absence of S-9 mix, it was repeated using the same conditions but with a highest concentration of 500 µg/plate and a total of five dose levels (5, 15, 50, 150 and 500 µg/plate).

Evaluation criteria: For a test to be considered valid, the mean of the solvent control revertant colony numbers for each strain should lie within the 99% confidence limits of the current historical control range of the laboratory. The positive control compounds must cause at least a doubling of mean revertant colony numbers over the negative control. The mean number of revertant colonies for all treatment groups was compared with those obtained for the solvent control groups. The mutagenic activity of a test substance was assessed by applying the following criteria:

(a) If treatment with a test substance produced an increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S-9 mix, it was considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

(b) If treatment with a test substance did not produce reproducible increases of at least 1.5 times the concurrent solvent controls, in either mutation test, it was considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.

(c) If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may have been performed in order to resolve the issue of the test substance's mutagenic activity in this test system. Should an increase in revertant colony numbers then be observed which satisfies paragraph (a), the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.

If no clear "positive" response was obtained, the test data may have been subjected to

analysis to determine the statistical significance of any observed increases in revertant colony numbers.

Findings:

In the first test, toxicity (visible thinning of the background lawn of non-revertant cells) was observed towards all the *S. typhimurium* strains at one or more concentrations. A maximum exposure concentration of 5000 µg/plate was therefore selected for use in the second test, except for strain TA1537, where 1500 µg/plate was selected for testing in the absence of S-9 mix.

In the second mutation test, toxicity was observed towards all tester strains at one or more concentrations. 500 µg/plate was therefore chosen as the top concentration in the third test.

In the third mutation test, no increases in revertant colony numbers over control counts were obtained with strains TA98, TA1535 and TA1537 following exposure to HYPA at any concentration in the absence of S-9 mix. Toxicity was obtained in all strains following exposure to HYPA at 500 µg/plate.

No evidence of mutagenic activity was seen at any dose level of HYPA in any mutation test. The concurrent positive controls demonstrated the sensitivity of the assay and the metabolizing activity of the liver preparations, inducing substantial increases in revertant colony numbers with all strains.

Conclusion:

HYPA showed no evidence of mutagenic activity in this bacterial system, either in the presence or absence of metabolic activation.

<u>Bacterial reverse mutation test of G-525 (MAPA):</u>

Reference.: *Ohtsuka M.; 1989;* Report No. T-1677E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

<u>The study is considered acceptable</u>.

Material and method:

This test was conducted to evaluate the mutagenic potential of G-525, MAPA (Lot No. 8805, purity 99.8%) in bacterial systems. The *S. typhimurium* (TA100, TA1535 and TA98) and *E. coli* (WP2 <u>uvr</u> A) tests were performed with 313, 625, 1250, 2500 and 5000 µg/plate in a DMSO solution without metabolic (S-9) activation and with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). For strain TA1537, tests were performed with 31.3, 62.5, 125, 250 500 and 1000 µg/plate without S-9 mix. With S-9 mix, the same dose levels as for the other bacterial strains were chosen. Dose levels were established on the basis of a preliminary range finding test.

Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for

48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.

Evaluation criteria:

The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose response.

Findings:

The results of the tests showed no increase in the number of revertant colonies at any dose level, either with or without metabolic activation for any bacteria tester strain exposed to G-525 (MAPA). AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).

Conclusion:

The results of the test indicate that G-525 (MAPA) showed no increase in the number of revertant colonies at any dose level.

G-450: Micronucleus test in male mice

Reference: *Inouye T.; 1989;* Report No. IET 89-0015

Guideline: The study was conducted according to Japanese MAFF, 59 NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This study was conducted to assess the potential induction of micronuclei by G-450 HYPA, (Lot No. 8805, purity 98.8%) in bone marrow cells of male mice.

6 male mice per group were treated with a single oral dose of HYPA (intragastric gavage) at dose levels of 100, 200 and 400 mg/kg bodyweight (in a preliminary toxicity test dose levels were chosen). Bone marrow smears were prepared 16, 24, 48 and 72 hours after administration of 400 mg/kg bw and 24 hours after administration of 100 and 200 mg/kg bw. The negative control group received the vehicle, 0.5 % carboxymethyl cellulose, and bone marrow smears were obtained from animals 16, 24, 48 and 72 hours after administration. The positive control group was dosed with mitomycin C at 2 mg/kg bodyweight and bone marrow smears were prepared once 24 hours after administration.

Three smears from each animal was examined using light microscopy. The frequencies of micronucleated polychromatic erythrocytes and the polychromatic erythrocyte ratios were analyzed. The frequencies of micronucleated polychromatic erythrocytes are shown as percentages of polychromatic erythrocytes with micronuclei among 2000 polychromatic erythrocytes. The polychromatic erythrocyte ratios as an indicator of hematopoesis are shown as percentages of polychromatic erythrocytes among 1000 erythrocytes scored.

Evaluation criteria:

The mean frequency of micronucleated polychromatic erythrocytes in the negative control group should be 0.3% or less. The mean frequency of micronucleated polychromatic

erythrocytes in the positive control group should be 2.0% or more.

A positive response is indicated if statistically significant dose-response increases in the frequency of micronucleated polychromatic erythrocytes are observed.

Findings:

At the highest dose of 400 mg/kg bw, mortality was 26 %. The frequencies of micronucleated polychromatic erythrocytes of the treatment groups were lower than the concurrent negative control values at any sampling time. 16, 24, 48 and 72 hours after dosing, the mean frequencies at 400 mg/kg bw were 0.08 %, 0.15 %, 0.06 % and 0.04 % respectively, the corresponding negative control values 0.27 %, 0.25 %, 0.22 % and 0.13 %. The mean frequencies at 100 and 200 mg/kg bw were 0.16 % and 0.13 %, respectively, the corresponding negative control value 0.25 %. In the positive control group (mitomycin C), an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes (3.88 %).

The ratios of polychromatic erythrocytes (PCEs) to all red blood cells (RBCs) at any dose and sampling time were slightly lower than the concurrent negative control values but were within the historical background values.

Table 6.8.1-1: Frequencies of micronucleated polychromatic erythrocytes (MNPCE/PCE) and polychromatic erythrocyte ratios (PCE/(PCE+NCE)

Dose (mg/kg)	Sampling time	MNPCE/PCE (%) (mean)	PCE/(PCE+NCE) (%) (mean)
Carboxymethyl cellulose	16	0.27	49.6
	24	0.25	51.4
	48	0.22	50.3
	72	0.13	58.2
100	24	0.16	42.8
200	24	0.13	46.3
400	16	0.08	48.5
	24	0.15	45.2
	48	0.06	48.4
	72	0.04	53.8
mitomycin C	24	3.88	43.3

MNPCE: micronucleated polychromatic erythrocytes
PCE: polychromatic erythrocytes
NCE: normochromatic erythrocytes

Conclusions:

From the results obtained, it is concluded that a single oral administration of G-450 does not induce micronuclei in the bone marrow cells of male mice under the conditions of this test.

G-450: Micronucleus test in female mice

Reference: *Inouye T.; 1989;* Report No. IET 89-0016

Guideline: The study was conducted according to Japanese MAFF, 59 NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This study was conducted to assess the potential induction of micronuclei by G-450 HYPA, (Lot No. 8805, purity 98.8%) in bone marrow cells of female mice.

6 female mice per group were treated with a single oral dose of HYPA (intragastric gavage) at dose levels of 143, 285 and 570 mg/kg bodyweight (in a preliminary toxicity test dose levels were chosen). Bone marrow smears were prepared 16, 24, 48 and 72 hours after administration of 570 mg/kg bw and 24 hours after administration of 143 and 285 mg/kg bw. The negative control group received the vehicle, 0.5 % carboxymethyl cellulose, and bone marrow smears were obtained from animals 16, 24, 48 and 72 hours after administration. The positive control group was dosed with mitomycin C at 2 mg/kg bodyweight and bone marrow smears were prepared once 24 hours after administration.

Three smears from each animal were examined using light microscopy. The frequencies of micronucleated polychromatic erythrocytes and the polychromatic erythrocyte ratios were analyzed. The frequencies of micronucleated polychromatic erythrocytes are shown as percentages of polychromatic erythrocytes with micronuclei among 2000 polychromatic erythrocytes. The polychromatic erythrocyte ratios as an indicator of hematopoesis are shown as percentages of polychromatic erythrocytes among 1000 erythrocytes scored.

Evaluation criteria:

The mean frequency of micronucleated polychromatic erythrocytes in the negative control group should be 0.3% or less. The mean frequency of micronucleated polychromatic erythrocytes in the positive control group should be 2.0% or more.

A positive response is indicated if statistically significant dose-response increases in the frequency of micronucleated polychromatic erythrocytes are observed.

Findings:

At the highest dose of 570 mg/kg bw, mortality was 7.4 %. The frequencies of micronucleated polychromatic erythrocytes of the treatment groups were not significantly higher than the concurrent negative control values at any sampling time. 16, 24, 48 and 72 hours after dosing, the mean frequencies at 570 mg/kg bw were 0.25 %, 0.20 %, 0.20 % and 0.12 % respectively, the corresponding negative control values 0.12 %, 0.23 %, 0.20 % and 0.15 %. The mean frequencies at 143 and 285 mg/kg bw were 0.15 % and 0.08 %, respectively, the corresponding negative control value 0.23 %. In the positive control group (mitomycin C), an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes (3.67 %).

The ratios of polychromatic erythrocytes (PCEs) to all red blood cells (RBCs) at any dose and sampling time were within the historical background values.

Table 6.8.1-2: Frequencies of micronucleated polychromatic erythrocytes (MNPCE/PCE) and polychromatic erythrocyte ratios (PCE/(PCE+NCE)

Dose (mg/kg)	Sampling time	MNPCE/PCE (%) (mean)	PCE/(PCE+NCE) (%) (mean)
Carboxymethyl cellulose	16	0.12	56.9
	24	0.23	61.1
	48	0.20	58.3
	72	0.15	56.8
143	24	0.15	62.8
285	24	0.08	63.1
570	16	0.25	58.5
	24	0.20	59.2
	48	0.20	62.4
	72	0.12	52.0
mitomycin C	24	3.67	51.5

MNPCE: micronucleated polychromatic erythrocytes
PCE: polychromatic erythrocytes
NCE: normochromatic erythrocytes

Conclusions:

From the results obtained, it is concluded that a single oral administration of G-450 does not induce micronuclei in the bone marrow cells of female mice under the conditions of this test.

B.6.8.2 Toxicity studies on impurities

Acute oral toxicity to rats of G-624:

Reference: *Liggett, M. P.; 1988;* Report No. 881247D/ISK20/AC

Guideline: The study was conducted according to Japanese MAFF Test Guidelines, 59 NohSan No. 4200 (1985).

GLP: yes

Material and Methods:

G-624, chemical name [redacted] is an impurity of technical fluazinam (Impurity 6).

Groups of 5 rats/sex (strain: CFY Sprague-Dawley; source: Interfauna UK Ltd., Huntingdon) weighing between 98 and 150 g (4 - 6 weeks old) received single oral doses of 3200, 4000, 5000 and 6400 mg/kg bw G-624 (Lot No. 8806, purity 99.6%).

G-624 was suspended as a w/v formulation in aqueous methylcellulose by gavage. After administration all animals were kept under observation for 15 days. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All mice were necropsied and macroscopic organ changes reported.

Findings:

Clinical signs and mortality:

Signs of reaction to treatment in all rats within 4 hours of treatment were pilo-erection, hunched posture, waddling and diarrhoe. Lethargy and pallor of the extremities was observed in all rats at a dose level of 4000 mg/kg bw G-624 and above. Increased salivation was seen in both sexes shortly after administration at dose levels of 3200 and 6400 mg/kg bw. Decreased respiratory rate was observed amongst rats treated at 6400 mg/kg bw. Recovery of surviving rats was complete by day 4 (3200 mg/kg bw) or from day 6 to day 8 (4000 mg/kg bw and above).

Treatment-related deaths were observed amongst both sexes dosed at 4000 mg/kg bw and above. Deaths occurred from day 2 until day 4 after dosing. To calculate LD_{50} values for males or combined sexes was not possible due to insufficient mortality (table 6.8.2.-1). However, it was possible to conclude that the acute oral median lethal dose (LD_{50}) of G-624 was greater than 5000 mg/kg bw.

Pathology:

No macroscopic organ changes were noted in mice that died or in any of the surviving animals at necropsy.

Table 6.8.2-1: Mortality data for mice dosed with G-624

Sex	Dose (mg/kg)	No. of deaths in a group of 5	Day					
			1	2	3	4	5	6-15
males	3200	0						
	4000	1					1	
	5000	0						
	6400	0						
females	3200	0						
	4000	1			1			
	5000	1				1		
	6400	3		2	1			

Conclusion:

Under the conditions of this study, the acute oral median lethal dose (LD_{50}) of G-624 in rats of both sexes observed for a period of 15 days was > 5000 mg/kg bw.

Bacterial reverse mutation test of G-624:

Reference.: *Ohtsuka M.; 1989;* Report No. T-1740E

Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.

The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of G-624, (Lot No. 8806, purity 99.6%) in bacterial systems. G-624, chemical name [redacted] is an impurity of technical fluazinam (Impurity 6).

Dose levels were established on the basis of a preliminary range finding test.

For *S. typhimurium* tester strains TA100 and TA1535 the selected dose levels were 0.1, 0.3, 1.0, 3.0, 10, 30 and 90 µg/plate in a DMSO solution without metabolic (S-9) activation and 1.0, 3.0, 10, 30, 100 and 300 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). For *S. typhimurium* tester strains TA98 and TA1537 dose levels were 12.5, 25, 50, 100, 200 and 400 µg/plate with and without metabolic (S-9) activation.

E. coli (WP2 uvr A) tests were performed at dose levels of 157, 313, 625, 1250, 2500 and 5000 µg/plate with and without metabolic (S-9) activation.

Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.

Evaluation criteria:

The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose response.

Findings:

The results of the tests showed a dose-dependent increase in the number of revertant colonies of more than twice that of the solvent control for all S. typhimurium strains tested with and without metabolic (S-9) activation and for *E. coli* (WP2 uvr A) in the absence of S-9 mix.

AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).

Conclusion:

The results of the test indicate that G-624 showed reverse mutagenicity against S. typhimurium TA98, TA100, TA1535 and TA1537 with and without S-9 mix and also against *E. coli* (WP2 uvr A) in the absence of S-9 mix at the concentrations tested.

[redacted] in Fluazinam technical: Toxicological effect on brain of mice following a single oral administration

Reference.: *Nomura M.; 1998;* Report No. AN-1375/1411/1486

Guideline: No specific test guideline is mentioned in the study.

The aim of this study was to assess the toxicological effects of [redacted] The study is considered as supplementary information.

GLP: The study is not in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060).

Material and method:

Three studies were conducted to assess the toxicological effects of [redacted] of fluazinam technical. Each of [redacted] of fluazinam was administered as a single oral dose (gavage) to a group of 5 male mice (strain: CD-1 (ICR); source: Charles River Japan). Doses between 5 and 200 mg/kg bw were selected, because fluazinam technical at a dose of 5000 mg/kg bw could contain [redacted] 200 mg/kg bw of [redacted] was additionally examined.

Table 6.8.2-2: Organization of the 3 studies:

Study number	Test material	Dose (mg/kg bw)	Number of animals
Animals were observed 5, 10 and 30 minutes and 1, 2, 3, 20, 24 and 48 hours AN-1375	Vehicle control (corn oil)	0	5
	[redacted]	50	5
	[redacted]	50	5
	[redacted]	50	5
	[redacted]	50	5
observed 5, AN-1411	Vehicle control (corn oil)	0	3
	[redacted]	20	5
	[redacted]	200	5
	[redacted]	50	5
	Impurity 6 [redacted]	50	5
	[redacted]	100	5
' AN-1486	Vehicle control (corn oil)	0	5
10	Impurity 5 [redacted]	5	5

Animals were observed 5, 10 and 30 minutes and 1, 2, 3, 20, 24 and 48 hours after dosing for clinical signs or reaction to treatment. The five mice receiving Impurity-5 were observed for 24 hours only and then terminated because of moribund state. Body weights were assessed on the first day of treatment and at necropsy. At necropsy, the brain from all animals was dissected at the regions between the olfactory bulb and cerebrum and between medulla oblongata and cervical spinal cord, observed macroscopically and weighed individually, followed by a histopathological examination.

Findings:

General observations: No deaths or clinical signs were noted in the mice receiving any of the test materials except Impurity-5. 3 animals receiving Impurity-5 exhibited coarse fur, paralysis of hind legs and staggering gait 20 hours after dosing and thereafter. The other 2 animals exhibited sedation. These 5 animals were killed 24 hours after dosing.

Body weights were statistically significantly lower than controls in 3 animals receiving Impurity-5. A statistically significant increase of brain weight was observed in all 5 mice of this group.

Macroscopic pathological changes related to treatment were confined to the brain of mice receiving Impurity-5. Trace to moderate edema of the brain was noted in 4 of 5 animals which received Impurity-5.

Histopathological examination of the brain revealed vacuolation of white matter in all Impurity-5 treated mice. Animals from the other groups showed no abnormalities compared to controls.

Conclusion:

Dietary administration of Impurity-5 to male mice at a concentration of 5 mg/kg bw caused clinical signs, decreased body weights, increased brain weights, edema of the brain and vacuolation of white matter in the brain. The oral administration of other [redacted] at a single oral dose of 50 mg/kg bw or higher caused no adverse effects on mortality, clinical signs, body and brain weight, macroscopic pathology or histopathology of the brain.

B-1457 (Impurity 5): Comparative Study on Susceptibility to Neurotoxicity in Mice, Rats and Dogs

Reference: *Nakashima N.; 1998;* Report No. IET 98-0020

No test guideline is mentioned in the study, nevertheless, it is in compliance with GLP. The study is considered as supplementary information.

Material and method:

Impurity 5 (Lot Nr. 9604, purity 97 %), chemical name [redacted] suspended in 0.5 % carboxymethylcellulose sodium, was administered to 5 male mice (strain: ICR (Crj: CD-1), 5 male rats (strain: Sprague-Dawley Crj:CD (SD)) and 3 male beagle dogs at a dose level of 2 mg/kg bw/d by gavage for a period of 3 days. The same number of animals of all 3 species received the vehicle only and served as controls. For dogs, the test substance was divided into halves and administered one hour apart to prevent vomiting.

All animals were observed daily for clinical signs or reaction to treatment. Body weights were recorded daily.

Approximately 24 hours after the last dosing, animals were necropsied and brain weight was measured. Histopathological examinations were performed on the brain and liver.

Findings:

There were no mortalities during the study period. On day 2 and thereafter, decreased

spontaneous motor activity was observed in 3 treated mice and all treated rats. Body weights of mice and rats were statistically significantly lower than controls on day 3 or 4 of treatment. At necropsy, absolute brain weight was significantly increased in treated mice and rats. Gross observations included swelling of the brain in all treated rats, a red spot on the lung of one rat and distended urinary bladders in 3 treated rats. Gross lesions were not observed in mice or dogs.

Histopathological examination of the brain revealed vacuolation of white matter in all treated mice, rats and dogs. In mice and rats, vacuolation was minimal and diffuse, the alteration in dogs trace and focal.

Conclusion:

The neurotoxic effect of Impurity 5 in mice and rats was comparable in quality and strength. In dogs, the magnitude and extent were only trace and focal. The lower susceptibility in dogs might be imputed to that further older dogs were not utilized. Adult mice and rats are more sensitive to neurotoxicity of Impurity 5 than pubescent ones. The two-divided administration method used for dogs to prevent vomiting might be another reason for the minor appearance of the brain histopathological alteration in dogs.

Impurity 5, an Impurity in Fluazinam technical: Toxicological Effect on Brain and Optic nerves of Mice following a single oral Administration at various stages of Animal Age

Reference: *Nomura M.; 1998;* Report No. AN-1480

No test guideline is mentioned in the study and it is not in compliance with GLP. The study is considered as supplementary information.

Material and method:

Eight groups of five male mice (strain: Crj:CD-1 (ICR)) each received a single oral dose of 2.5 mg/kg of Impurity-5 (Lot Nr. Y950807, purity 99.5%) by gastric intubation in an aqueous solution of 0.5% carboxymethyl cellulose sodium salt (CMC-Na). The groups were 3, 5, 8, 10, 12, 16, 20 and 24 weeks old, respectively, when they received the dose. Observations for clinical signs and mortality were made frequently within three hours and at 19, 24 and 48 hours after dosing. Individual body weight was recorded at dosing and at termination. All the animals were killed 48 hours after dosing and the animals were subjected to necropsy, brain weight measurement and a histopathological examination of the brain and optic nerves with eyes.

Findings:

There were no clinical signs and body weight increased with animal age. Brain weights were considered to be normal, but no data was available for untreated controls. There were no macroscopic findings except for one Group 4 animal (10 weeks old at dosing) which showed edema in the brain. Vacuolation of white matter in the brain was observed in groups 1 to 3 (3, 5 and 8 weeks old) at the severity of “trace” with the incidence increasing with age. The same finding was observed in all animals at the severity of “trace” or “minimal” in groups 4 to

8 (10, 12, 16, 20 and 24 weeks old). Vacuolation in the optic nerves was observed in all groups except group 1 at the severity of "trace", but the effect was less severe than that of the brain. No abnormal findings were observed in the eyes of any animal.

Conclusion:

Based on the results obtained in the study, the effects of Impurity-5 on the brain and optic nerves of mice were considered to increase with animal age until about ten weeks and then remain at a constant level until at least 24 weeks.

Impurity 5, an Impurity in Fluazinam technical: Sensitivity Comparison on Brain of Mice and Rats following 14 day Oral Administrations

Reference: *Nomura M.; 1998;* Report No. AN-1481

No test guideline is mentioned in the study and it is not in compliance with GLP. The study is considered as supplementary information.

Material and method:

A group of seven female mice (strain: Crj:CD-1 (ICR) SPF/VAF) and seven female rats (strain: Crj:CD (SD) SPF/VAF) received Impurity-5 (Lot Nr. Y950807, purity 99.5%) at a concentration of 0.5 mg/kg bw/d by gastric intubation for 14 consecutive days. A control group of each species was treated with the vehicle alone, 0.5% carboxymethyl cellulose sodium salt (CMC-Na). Clinical signs and mortality were observed daily. Individual body weight was measured before each of the fourteen-day administrations and at necropsy. Twenty-four hours after the last test material administration, all the animals were subjected to necropsy including weighing and histopathological examination of the brain.

Findings:

There were no deaths and no clinical signs indicative of a reaction to treatment throughout the study for all groups, and there were no abnormalities for all groups at the macroscopic examination. The microscopic examination of the mice and rats showed similar sensitivity to the vacuolation of the white matter of the brain.

Conclusion:

After 14 days of treatment with Impurity-5, female rats and mice showed similar sensitivity to the vacuolation of the white matter of the brain.

Impurity 5, an Impurity in Fluazinam technical: Sensitivity Comparison on Brain of Rats and Mice in three and 10 weeks old following 14 day Oral Administrations

Reference: *Nomura M.; 1998;* Report No. AN-1492

No test guideline is mentioned in the study and it is not in compliance with GLP. The study is considered as supplementary information.

Material and method:

A group of five male mice (strain: Crj:CD-1 (ICR) SPF/VAF) and five male rats (strain: Crj:CD (SD) SPF/VAF), 3 weeks old, and a group of five male mice and five male rats, 10 weeks old, received Impurity-5 (Lot Nr. Y950807, purity 99.5%) at a concentration of 0.5 mg/kg

bw/d by gastric intubation for 14 consecutive days. Control groups of each species were treated with the vehicle alone, 0.5% carboxymethyl cellulose sodium salt (CMC-Na). Clinical signs and body weight were noted daily prior to each administration and at necropsy. All the animals were subjected to a necropsy 24 hours after the final administration of the test material that included weighing and histopathological examination of the brain.

Findings:

All animals survived the treatment period. There were no clinical signs indicative of treatment and body weights were comparable to controls. The brain of one rat and two mice in the ten-week old groups were observed with edema at the macroscopic necropsy examination, but there were no statistically significant differences in mean brain weights in the treated groups as compared to the control groups. At microscopic examination, the rats and mice showed the same sensitivity for the vacuolation of the white matter of the brain related to impurity-5 administration. The 10-week old animals were slightly more sensitive than the 3-week old animals in both species.

Conclusion:

The incidence and severity of white matter vacuolation of the brain is similar in male mice and rats, but ten-week old animals were more sensitive in both rats and mice as compared to the three-week old animals.

B.6.8.3 General Pharmacology

Effects on biological function of fluazinam technical (General pharmacology)

Reference: *Maebashi H.; 1988;* Report No. FR-2501

Guideline: The study was conducted according to Japanese MAFF, 59 NohSan No. 4200 (1985) and is in compliance with GLP.

The study is of supplementary information.

Material and methods:

For all tests performed, Technical fluazinam, Lot No. 109, purity 95.3%, was used.

General condition test: Fluazinam, dissolved in 0.5% w/v aqueous carboxymethylcellulose at concentrations of 0, 10, 20, 40, 80, 160 and 320 mg/kg bw, was administered intraperitoneally (i.p.) at 10 ml/kg bw to groups of 3 male and 3 female ICR-mice each. The general condition was observed by the method of multiple observation of Irwin. The objects of the observation were recognizability, exercise, central erethism, posture, exercise loss, reflection, autonomic nervous syndrome, life and death and other symptoms. Before administration, 15, 30, 60 minutes, 2, 4, 6, 24, 48, 72 and 96 hours after administration, conditions were observed.

Brain wave test: Fluazinam, dissolved in polyethylene glycol at concentrations of 0, 0.1, 0.5, 1.0 and 2.0 mg/kg bw, was administered intravenously (i.v.) to 3 male Japanese white rabbits increasingly every 30 minutes.

Respiration and circulation test: Concentrations of 0, 0.1, 0.2, 0.5, 1.0 and 2.0 mg/kg bw fluazinam, dissolved in polyethylene glycol, were administered intravenously (i.v.) every 30

minutes to 3 male Japanese white rabbits. For breathing exercise, a respiratory pressure transducer was used. For blood pressure, carotid artery pressure was measured by the transducer for the measurement of blood pressure through vein cannula. Pulsation was counted through electroradiogram.

Body temperature and action of pupil test: 0, 0.25, 0.5 and 1.0 mg/kg bw fluazinam, dissolved in polyethylene glycol, were administered intravenously (i.v.) to groups of 3 male Japanese white rabbits. Body temperature was measured before administration, 30 minutes, 1, 2 and 3 hours after administration. For the action of pupil test, illuminant powder was streamed to both eyes before i.v. administration of fluazinam. 5, 15, 30 and 60 minutes after administration of fluazinam, the diameter of the pupils was measured.

Action of transportability of small intestine: The action of fluazinam on intestinal transportability of rats was examined following subcutaneous injection of 0, 625, 1250, 2500 and 5000 mg/kg bw to groups of 10 animals each.

Tibialis anterior muscle contraction: 0, 0.2, 0.5, 1.0 and 2.0 mg/kg bw fluazinam, dissolved in polyethylene glycol, were administered intravenously (i.v.) to 3 male Japanese white rabbits every 30 minutes. Indirect muscle stimulation was done through bipolar platinum electrode which was connected to the common peroneal nerve, direct muscle stimulation was done by using platinum electrode which was touched to the vein.

Action of Hemolysis: Fluazinam, dissolved in physiological saline at concentrations of 10-5, 5x10-5, 10-4, 5x10-4 and 10-3, was added to the red blood cells floating solution (prepared from blood taken from rabbits, washed three times with physiological saline, and floated in a 10-fold volume of physiological saline) and incubated at 38o C for 2 hours. Then the mixture was centrifuged and the absorption of the supernatant measured at 540 nm by spectrum photometer. The value of hemolysis in distilled water was assumed to be 100 %, in physiological saline 0 % and the rateof hemolysis was represented by percentage.

Findings:

General condition test: At doses of 160 mg/kg and less, there were no changes observed in male mice. At 320 mg/kg, decrease in body temperature occurred after 48 hours in one mouse, which died after 72 hours. At doses of 80 mg/kg and less, there were no changes observed in female mice. At 160 mg/kg, decrease in body temperature occurred after 48 hours. At 320 mg/kg, one mouse died at 24 hours, one at 48 hours and one at 72 hours.

Brain wave test: No change was observed at both cortical and deep brain waves after administration of 0.1 mg/kg bw fluazinam. After administration of 0.5 mg/kg bw fluazinam, a decrease of the number of pulse was observed. The amplitude of both cortical and deep brain waves declined following administration of 1.0 mg/kg but a recovery was seen after 30 minutes. At 2.0 mg/kg, the rabbits died.

Respiration and circulation test: There was a dose dependent temporary rise in blood pressure and a decrease in pulse. At a dose of 1.0 mg/kg, there was a temporary rise in both the amplitude of respiration and breathing, which recovered after 30 minutes. After administration of 2.0 mg/kg, the rabbit died within a few minutes.

Body temperature and action of pupil test: After i.v. injection of 0.25, 0.5 and 1.0 mg/kg bodyweight, no changes in body temperature were seen. There were no significant changes in the pupil after dosing compared to before dosing.

Action of transportability of small intestine: Following subcutaneous injections of 0, 625, 1250, 2500 and 5000 mg/kg bodyweight, doses of 2500 mg/kg and less caused no observable changes in carbon powder transportability in the small intestine. A significant deterioration ($P < 0.05$) in transportability was observed following administration of the high dose.

Tibialis anterior muscle contraction: There was no effect on muscle contraction by direct or indirect stimulation following intravenous administration of 0.2, 0.5 or 1.0 mg/kg bw fluazinam. At 2.0 mg/kg, the rabbit died shortly after dose administration.

Action of Hemolysis: The result of the action of hemolysis by adding fluazinam to the red blood cells floating solution indicated that no hemolysis of red blood cells occurred by the addition of solutions of 0.5 mg fluazinam/ml or less. Addition of 1 mg/ml caused a slight action of hemolysis.

Conclusions:

At intraperitoneal doses of 80 mg/kg bw fluazinam technical and less, there were no changes in the condition of male or female mice. Though no death was caused in the groups of 160 mg/kg bw, a descent of body temperature was seen. Mice of the highest dose group (320 mg/kg bw) died without showing special symptoms expect the descent of body temperature. After administration of 0.5 mg/kg bw fluazinam i.v., a decrease of the number of pulse was observed. There were no effects on brain waves following i.v. administration of 0.5 mg/kg bw fluazinam. Following administration of 1.0 mg/kg bw, both cortical and deep brain waves declined but recovery was seen after 30 minutes. A dose dependent temporary rise in blood pressure and a decrease in pulse were observed in rabbits at a dose of 1.0 mg/kg bw fluazinam i.v., with recovery after 30 minutes. No change in the electrocardiogram and no effect on the pupils nor the contraction of tibialis anterior muscle of rabbits was observed. Suppression in carbon powder transportability in the small intestine was observed following subcutaneous administration of a high dose of 5000 mg/kg bw fluazinam. Doses of 2500 mg/kg and less caused no observable changes. Slight hemolysis was observed in erythrocytes of rabbits by adding fluazinam at a concentration of 1 mg/ml.

Fluazinam technical: Toxicological effect on brain of rats and its reversibility by dietary administration for 14 days followed by a 25 day recovery period

Reference.: *Nomura M.; 1998;* Report No. AN-1323

Guideline: No specific test guideline is mentioned in the study.

The aim of this study was to assess the toxicological effects of fluazinam on the brain of rats and to determine the reversibility of any such effects. Due to the restricted extent of parameters investigated, the study is considered as supplementary information.

GLP: The study is not in accordance with LSR Standard Operating Procedure Quality

Assurance Unit (QAU/060).

Material and method:

Three groups of 7 male rats (strain: CD rats (remote Sprague-Dawley); source: Charles River Japan) received a diet containing 0, 10000 and 30000 ppm fluazinam technical (Lot Nr. 1030/91, purity 96.2 %), equivalent to 0, 714 and 1743 mg/kg bw/d. After 14 days 4 rats of each group were killed and subjected to the terminal examination. 3 animals from each group were maintained for an additional 25 days, without treatment, in order to assess the reversibility of any treatment-related effect.

Animals were observed daily for clinical signs or reaction to treatment. Food consumption and body weights were assessed on the first day of treatment, twice a week thereafter and five times during the recovery period. At necropsy, the brain from all animals was dissected at the regions between the olfactory bulb and cerebrum and between medulla oblongata and cervical spinal cord, observed macroscopically and weighed individually, followed by a histopathological examination.

Findings:

General observations: There were 3 deaths, one found dead and two killed in extremis, during days 12 to 14 of treatment in the 30000 ppm group. Animals had shown decreased locomotor activity and two of them had signs of anemia before death. Coarse fur was observed in all treated rats from day 4 or 5 throughout the treatment period. Emaciation was observed on days 5 and 6 in the 30000 ppm group.

Food consumption and body weights of treated animals were statistically significantly lower than controls throughout the treatment period. After the recovery period body weights were similar to controls.

Macroscopic pathological changes related to treatment were confined to the brain and liver. A trace grade of edema was observed only in the brain of rats receiving 30000 ppm. Enlarged livers with accentuated lobular patterns and pale discoloration were observed as a minimal grade at 10000 ppm and as moderate at 30000 ppm. No macroscopic pathological changes were observed in the animals killed at the end of the recovery period.

Histopathological examinations of the brain revealed vacuolation of white matter in all treated rats. The severity of this finding was trace at 10000 ppm and minimal to moderate at 30000 ppm. In rats killed after the recovery period, a trace grade of vacuolation of white matter remained only in the animals of the 30000 ppm group. No abnormal findings were observed in animals of the 10000 ppm group.

Conclusion:

Dietary administration of fluazinam at higher concentrations caused vacuolation of white matter in the brain. The affected rats recovered nearly completely after a 25 day recovery period, indicating that the histopathological change is reversible.

Fluazinam technical: Toxicological effect on brain of mice and its reversibility by dietary administration for 4 or 28 days followed by a 56 day recovery period

Reference.: *Nomura M.; 1998;* Report No. AN-1333

Guideline: No specific test guideline is mentioned in the study.

The aim of this study was to assess the toxicological effects of fluazinam on the brain of mice and to determine the reversibility of any such effects. Due to the restricted extent of parameters investigated, the study is considered as supplementary information.

GLP: The study is not in accordance with LSR Standard Operating Procedure Quality Assurance Unit (QAU/060).

Material and method:

Fluazinam technical (Lot Nr. 1030/91, purity 96.2 %) was administered to 13 groups of 5 male mice per group (strain: CD-1 (ICR); source: Charles River Japan) at dietary concentrations of 7000 and 20000 ppm for 4 or 28 days.

Five groups received a diet containing 7000 ppm (equivalent to 1173 mg/kg bw/d) and five groups received 20000 ppm (equivalent to 1871 mg/kg bw/d) for 4 days. Following the 4 day treatment period, one group at each dose level was terminated immediately and 4 of the 5 groups from each dose level were maintained for a recovery period of 7, 14, 24 or 56 days. 5 groups of mice were designated as controls and subjected to the same schedule as the treated groups.

3 groups of mice received 7000 ppm fluazinam (equivalent to 1043 mg/kg bw/d) for 28 days. 2 of the 3 groups were maintained for a recovery period of 28 or 56 days and the other group was terminated immediately following the 28 day treatment period. 3 groups of mice were designated as controls and subjected to the same schedule as the treated groups.

Animals were observed daily for clinical signs or reaction to treatment. In the groups treated for 4 days, food consumption and body weights were assessed on the first day of treatment and on day 7, 14, 21, 24, 31, 38, 45, 52 and/or 56 of recovery. Food consumption and body weights were assessed on the first day of treatment and once a week during the treatment and recovery period in the groups treated for 28 days.

Selected groups of mice were scheduled for neurobehaviorial assessments using a functional observational battery (FOB) or for in situ perfusion for electron microscopic evaluation.

At necropsy, the brain from all animals was dissected at the regions between the olfactory bulb and cerebrum and between medulla oblongata and cervical spinal cord, observed macroscopically and weighed individually, followed by a histopathological examination.

Findings:

General observations: No deaths or clinical signs indicative of neurological effects were noted. Abnormal FOB findings such as hunched posture, impaired mobility, straub tail, landing foot splay wider than controls were noted in one group of mice receiving 20000 ppm for 4 days. Abnormal findings were only observed on day 4 of treatment, not afterwards during the recovery period.

Food consumption was decreased in animals receiving 20000 ppm, but was comparable to controls within the first week of recovery period. Body weights were statistically significantly lower than controls in 7000 ppm animals treated for 28 days and in 20000 ppm animals treated for 4 days. Body weights were similar to controls by day 7 or 14 of the recovery period.

Macroscopic pathological changes related to treatment were confined to the brain and liver. Edema of the brain was observed in mice receiving 7000 ppm for 28 days and 20000 ppm for 4 days. The edema was reversed by day 7 of the recovery period.

Enlarged livers with accentuated lobular patterns and pale discoloration were observed in all treatment groups. The liver findings were reversed by day 7 of the recovery period.

Histopathological examinations of the brain revealed vacuolation of white matter in all treated mice terminated immediately following exposure. The vacuolation of white matter in the brain was reversed by day 24 for the animals treated at 7000 ppm for 4 days and by day 56 for animals treated at 7000 ppm for 28 days or at 20000 ppm for 4 days. Representative electron-microscopic photographs of the cerebellum white matter indicated that the effect of treatment appeared to be confined to the myelin sheaths, that the nucleus and mitochondria in oligodendroglia were kept intact and that myelin sheaths had recovered during the recovery period.

Conclusion:

Dietary administration of fluazinam caused vacuolation of white matter in the brain of mice. This effect was confined to the myelin sheaths and it was considered to be reversible.

Fluazinam: Overview Document on CNS Toxicological Finding due to an Impurity 5 in Fluazinam technical

Reference: *Nomura M.; 1998*

The purpose of this document is to provide an overview on a new toxicological finding caused by a single impurity (Impurity-5) present in fluazinam technical.

A thorough review of the regulatory and research studies conducted on the etiological factor determining the appearance of white matter vacuolation in the CNS has revealed the following:

Mice, rats and dogs histopathologically showed white matter vacuolation in the CNS only when given high doses of fluazinam technical without related clinical signs, so it was considered possible that an impurity, not fluazinam itself, is responsible for white matter vacuolation. So the toxicological effect on brain of mice following a single oral administration of [redacted] in fluazinam technical was tested: Impurity-5 at a concentration of 5 mg/kg bw caused increased brain weights, edema of the brain and vacuolation of white matter in the brain of this mice. The oral administration of [redacted] at a concentration of 50 mg/kg bw or higher caused no adverse effects on brain weight, macroscopic pathology or histopathology of the brain (*Nomura M.; 1998;* Report No. AN-1375/1411/1486). So fluazinam itself did not induce white matter vacuolation in the CNS of

animals.The appearance of white matter vacuolation in the CNS at high doses of fluazinam technical was dependent upon the presence of Impurity-5.

Different Lot Numbers of fluazinam technical used in toxicological studies were analyzed by HPLC for its concentration of Impurity-5. The results of the analyses showed that in those studies in which high doses of fluazinam technical failed to induce white matter vacuolation in the CNS, the batch contained very low levels of Impurity-5 (< 0.005 %). In order to investigate the threshold value for white matter vacuolation in the CNS induced by Impurity-5, the available studies were reanalyzed to calculate the achieved daily intake of Impurity-5 using its content in fluazinam technical and the fluaziname intake in each study of the mouse, rat and dog (table 6.7-1).

Table 6.8.3-1: Intake of Fluaziname and Impurity-5 in the studies of mice, rats and dogs and its relevance to white matter vacuolation in the CNS

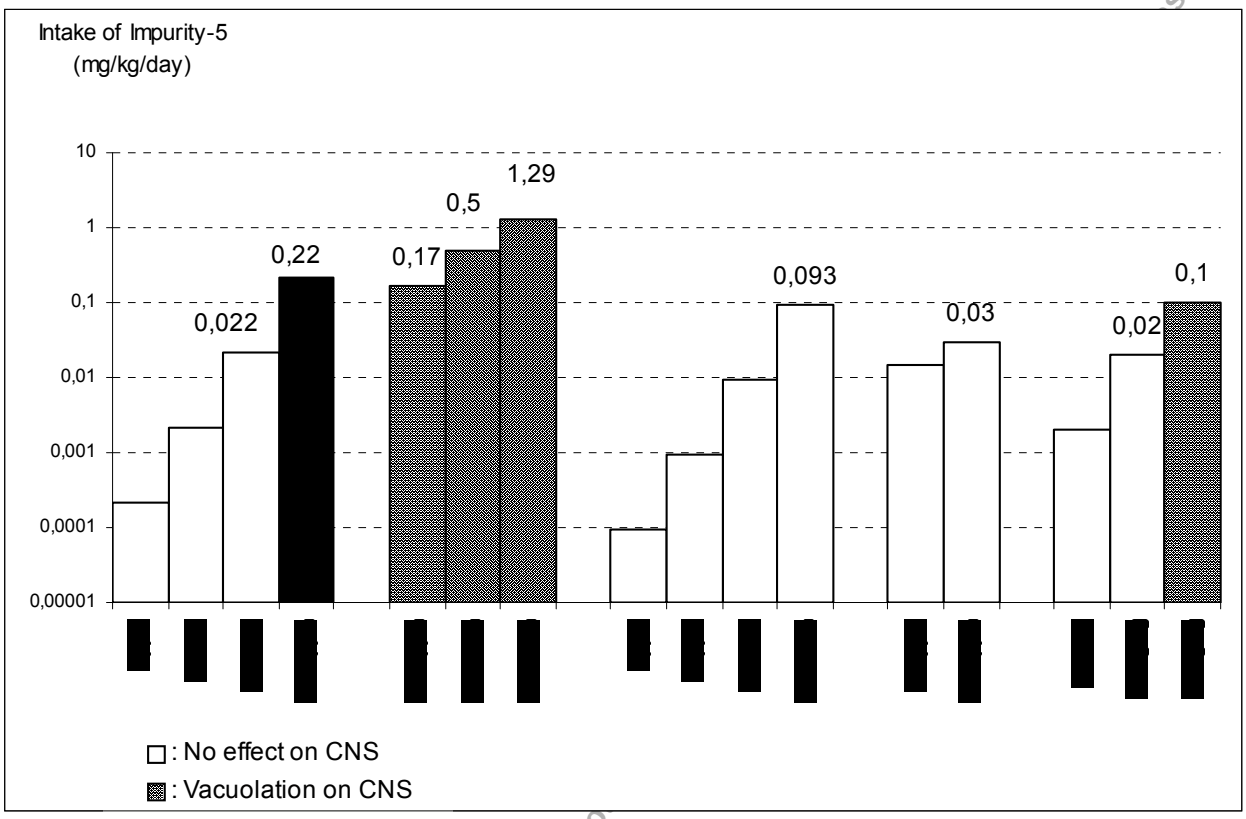
4-week toxicity study in mice (83/ISK036/067) Lot No. 8203, Impurity 5: 0.008%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	10 1.6 0.00013 -	50 7.9 0.00063 -	250 39.5 0.0032 -	3000 455 0.036 -	
4-week toxicity study in mice (ISK49/921049) Lot No. 1030/91, Impurity 5: 0.12%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	3000 607 0.73 +	5000 994 1.19 +	7000 1302 1.56 +		
2 year toxicity study in mice (ISK9/87264) Lot No. 8412-20, Impurity 5: 0.20%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	0 0 0 -	1 0.12 0.00023 -	10 1.14 0.0023 -	100 11.2 0.022 -	1000 112 0.22 +
2 year toxicity study in mice (ISK50/950671) Lot No. 1030/91, Impurity 5: 0.12%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	1000 144 0.17 +	3000 415 0.50 +	7000 1075 1.29 +		
4-week toxicity study in rats (82/ISK035/544) Lot No. 8203, Impurity 5: 0.008%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	10 1.26 0.0001 -	50 5.21 0.0004 -	250 26.1 0.0021 -	3000 305.4 0.024 -	
13-week toxicity study in rats (84/ISK046/635) Lot No. 8303, Impurity 5: < 0.005%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	2 0.16 <0.000008 -	10 0.82 <0.00004 -	50 4.1 <0.00021 -	500 42 <0.0021 -	

13-week neurotoxicity study in rats (ISK251/971800) Lot No. 6109, Impurity 5: 0.04%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	300 22.1 0.0088 -	1000 75 0.03 -			
2 year toxicity study in rats (ISK8/87263) Lot No. 8412-20, Impurity 5: 0.20%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	1 0.05 0.00001 NE	10 0.43 0.00085 NE	100 4.35 0.0087 NE	1000 46.5 0.093 -	
2 generations toxicity study in rats (87/ISK068/097) Lot No. 8412-20, Impurity 5: 0.20%						
fluazinam (ppm) **fluazinam (mg/kg/d)** **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 0 -	20 1.71 0.0034 -	100 5.66 0.011 -	500 42.0 0.084 -		
13-week toxicity study in dogs (84/ISK048/692) Lot No. 8303, Impurity 5: < 0.005%						
fluazinam (mg/kg/d) **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 -	1 <0.00005 -	10 <0.0005 -	100 <0.005 -		
52-week toxicity study in dogs (86/ISK055/512) Lot No. 8412-20, Impurity 5: 0.20%						
fluazinam (mg/kg/d) **Impurity 5 (mg/kg/d)** **CNS vacuolation**	0 0 -	1 0.002 -	10 0.02 -	50 0.1 +		

-: not observed; +: observed; NE: not examined

0.1 mg/kg bw/d of Impurity-5 was the lowest effect level for white matter vacuolation seen in the 52-week study in dogs. No vacuolation was seen in any species at lower dose levels. Therefore, a clear threshold dose has been established, which is independent of the levels of Impurity-5 in fluazinam technical. The CNS effect was dependent on the dose of Impurity-5 received by experimental animals. Expressing the data graphically (Fig. 6.7-1), it can be seen that no white matter vacuolation is observed at dose levels of Impurity-5 below approximately 0.1 mg/kg bw/d. The data support a non-linear dose-response for the production of white matter vacuolation in all of the 3 species with a threshold, below which no white matter vacuolation occurs, at approximately 0.1 mg/kg bw/d of Impurity-5.

Figure 6.8.3-1: Achieved intake of Impurity-5 expressed as a function of the presence or absence of white matter vacuolation



	ISK9/87264 Mouse 2 Years	ISK50/950671 Mouse 2 Years	ISK8/87263 Rat 2 Years	ISK251/971800 Rat 13 Weeks	ISK055/512 Dog 1 Year
Imp. 5 (%):	0.2 %	0.12 %	0.2 %	0.04 %	0.2 %
Batch no.:	8412-20	1030/91	8412-20	6109	8412-20

B.6.8.4 Summary of further toxicological studies

Metabolites:

The metabolite G-450, HYPA, chemical name 5-((3-chloro-5-(trifluoromethyl)-2-pyridyl)amino)- α,α,α- trifluoro-4,6-dinitro-o-cresol, detected in liver, kidney, muscle, fat and eggs of Laying hens but not in rat metabolism studies, was found to be more toxic than fluazinam after oral administration. The acute oral median lethal dose (LD_{50}) of HYPA in mice was 331 mg/kg bw (fluazinam is of low acute toxicity with LD50 values ≥ 4100mg/kg bw in mice and rats).

In an Ames- test, HYPA showed slight reverse mutagenicity against S. typhimurium TA98 without S-9 mix. The other bacteria tester strains (S. typhimurium TA100, TA1535, TA1537 and E.coli WP2uvrA) showed no increase in the number of revertant colonies at any dose level. In a second Ames-test, carried out with the same tester strains, HYPA showed no evidence of mutagenic activity, either in the presence or absence of metabolic activation. Micronucleus tests showed that HYPA does not induce micronuclei in the bone marrow cells of male and female mice.

Metabolite G-525, MAPA, chemical name 2-(2-amino-3-chloro-α,α,α-trifluoro-6-nitro-*p*-

toluidino)-3-chloro-5-(trifluoromethyl)pyridine was found in liver, kidney, muscle, fat and eggs of Laying hens but not in rat metabolism studies and showed only low acute toxicity with a LD_{50} > 5000 mg/kg bw in mice.

In a bacterial reverse mutation test, MAPA showed no increase in the number of revertant colonies at any dose level.

Impurities:

Impurity G-624, chemical name [redacted] showed low acute oral toxicity in rats of both sexes with a median lethal dose (LD_{50}) of > 5000 mg/kg bw. In a bacterial reverse mutation test, G-624 showed reverse mutagenicity against S. typhimurium TA98, TA100, TA1535 and TA1537 with and without S-9 mix and also against *E. coli* (WP2 uvr A) in the absence of S-9 mix. However, G-624 is included in toxicity studies with the active ingredient. There was no potential of genotoxicity seen with fluazinam technical, so no further studies with G-624 are required.

Dietary administration of Impurity-5, chemical name [redacted] to male mice at a concentration of 5 mg/kg bw caused clinical signs, decreased body weights, increased brain weights, edema of the brain and vacuolation of white matter in the brain. The oral administration of other 8 impurities at a single oral dose of 50 mg/kg bw or higher caused no adverse effects on mortality, clinical signs, body and brain weight, macroscopic pathology or histopathology of the brain.

The neurotoxic effect of Impurity 5 in mice and rats was comparable in quality and strength. In dogs, the magnitude and extent were only trace and focal. The lower susceptibility in dogs might be imputed to that further older dogs were not utilized. Adult mice and rats are more sensitive to neurotoxicity of Impurity 5 than pubescent ones. The two-divided administration method used for dogs to prevent vomiting might be another reason for the minor appearance of the brain histopathological alteration in dogs.

Based on the results obtained in the study, the effects of Impurity-5 on the brain and optic nerves of mice were considered to increase with animal age until about ten weeks and then remain at a constant level until at least 24 weeks.

After 14 days of treatment with Impurity-5, female rats and mice showed similar sensitivity to the vacuolation of the white matter of the brain.

The incidence and severity of white matter vacuolation of the brain is similar in male mice and rats, but ten-week old animals were more sensitive in both rats and mice as compared to the three-week old animals.

General Pharmacology:

At intraperitoneal doses of 80 mg/kg bw fluazinam technical and less, there were no changes in the condition of male or female mice. Though no death was caused in the groups of 160 mg/kg bw, a descent of body temperature was seen. Mice of the highest dose group (320 mg/kg bw) died without showing special symptoms expect the descent of body temperature. After administration of 0.5 mg/kg bw fluazinam i.v., a decrease of the number of pulse was observed. There were no effects on brain waves following i.v. administration of 0.5 mg/kg bw

fluazinam. Following administration of 1.0 mg/kg bw, both cortical and deep brain waves declined but recovery was seen after 30 minutes. A dose dependent temporary rise in blood pressure and a decrease in pulse were observed in rabbits at a dose of 1.0 mg/kg bw fluazinam i.v., with recovery after 30 minutes. No change in the electrocardiogram and no effect on the pupils nor the contraction of tibialis anterior muscle of rabbits was observed. Suppression in carbon powder transportability in the small intestine was observed following subcutaneous administration of a high dose of 5000 mg/kg bw fluazinam. Doses of 2500 mg/kg and less caused no observable changes. Slight hemolysis was observed in erythrocytes of rabbits by adding fluazinam at a concentration of 1 mg/ml.

The reversibility of vacuolation of white matter in the brain was tested after dietary administration of fluazinam at high concentrations (714 and 1743 mg/kg bw in rats; 1043, 1173 and 1871 mg/kg bw mice). The affected animals recovered nearly completely after a 25 day or 56 day recovery period respectively, indicating that the histopathological change is reversible.

Representative electron-microscopic photographs of the cerebellum white matter indicated that the effect of treatment appeared to be confined to the myelin sheaths, that the nucleus and mitochondria in oligodendroglia were kept intact and that myelin sheaths had recovered during the recovery period.

In studies in which high doses of Fluazinam technical failed to induce white matter vacuolation in the CNS, the batch contained very low levels of Impurity-5 (< 0.005 %).The threshold dose was independent of the Impurity-5 level in fluazinam technical. The CNS effect depends on the dose of Impurity-5 received by experimental animals. No white matter vacuolation was observed in rats, mice and dogs at dose levels of Impurity-5 below approximately 0.1 mg/kg bw/d.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Plant personnel

Shortly after commercial production of Fluazinam technical commenced, an incident of allergic contact dermatitis of a 20 year old worker involved in the milling process was reported in Japan in 1990 (Tominaga, K., Imamura, T. Nishioka, K., Asagami, C.: Systemic Contact Dermatitis due to Fluazinam; Skin Research 1991:33 (suppl 11) 364-368). The patient, who had erythema in the face and arms, tested positive in the patch test. Blood parameters showed increased levels of GOT, GPT, LDH and eosinophils (18 %). Based on the clinical findings and the results of the patch test, a systemic contact dermatitis due to fluazinam was diagnosed.

B.6.9.2 Direct observations

Data on direct observations is available for the Netherlands where Fluazinam is most widely and extensively used on potato crop. Fluazinam formulation was introduced on the Dutch

market in 1992 to protect potatoes and lily bulbs against molds. Ten farmers dealing with lilies or seed potatoes experienced adverse effects on the skin some weeks after initial contact with fluazinam. Seven of the nine farmers showed positive in patch tests with the formulation and with fluazinam itself (*van Ginkel, C.J.W., Sabapathy, N.N.: Allergic contact dermatitis from the newly introduced fungicide fluazinam. Contact Dermatitis 1995:32 160-162.).* To minimize incidences as much as possible, farmers were supplied with detailed information about the hazards of the fungicide.

There was an outbreak of contact dermatitis in a tulip bulb processing company in the Netherlands shortly after introduction of the fungicide fluazinam in 1993 (*Bruynzeel, D.P., Tafelkruijer, J., Wilks, M.F. Contact dermatitis due to a new fungicide used in the tulip bulb industry. Contact Dermatitis 1995:33 8-11.).* The eight employees showed positive patch tests. The dermatitis disappeared quickly when they stopped work, but returned as soon as they restarted work. Subsequent investigations showed that the fungicide had not been used according to the manufacturer's recommendations. The company introduced preventive measures and no new cases developed. The most sensitive employees could work under the new conditions without incidence.

Approximately 2,600 farmers in 1992 and 10,000 farmers in 1993 used fluazinam. About 0.19% of users reported adverse effects in 1992 and 0.36% in 1993. This rise in incidence is believed to be more a reflection of under-reporting in 1992, when the product was first used in potatoes, rather than a real increase. In the reported cases, there appeared to be a delayed type hypersensitivity reaction (type IV allergic reaction).

Despite label warnings, it was apparent that a proportion of affected individuals had not taken notice of label recommendations. As a result, label changes were made coupled with farmer education programs to minimize the incidence of sensitization to fluazinam. A program of continued stewardship and vigilance was introduced and incidence surveys are continuing.

The number of new incidences decreased from 89 in 1993 to a few cases per year. This analysis confirms that the trend in the number of newly occurring symptoms in the Netherlands has shown a dramatic decrease. The very small number of incidents confirms that the product is being used safely and without adverse effects by the overwhelming majority of users. Nevertheless, a program of continued stewardship and vigilance is being maintained.

B.6.9.3 Observations on exposure of general population and epidemiological studies

No reports on exposure of general population and epidemiological studies have been submitted.

B.6.9.4 Diagnosis of poisoning

The notifier refers to points 6.9.1 and 6.9.2 for signs, symptoms and results of clinical testing. Toxicity testing in animals for lethality demonstrated that fluazinam has a low degree of

acute toxicity by the oral and dermal routes. Via the inhalation route, technical fluazinam is classified as toxic. The median lethal concentration (LC_{50}) of the formulation via inhalation was greater than the maximum achievable concentration of the formulation ((LC_{50} > 1.15 mg/l).

Fluazinam technical caused severe eye irritation in rabbits and both the technical and formulated material showed potential for dermal sensitization.

B.6.9.5 Proposed first aid

The following first aid measures have been provided by the notifier:

- Treat symptomatically
- Initial measures:
  Initial measures would include establishment of a patent airway, removal of secretions, and respiratory resuscitation as needed.
- Decontamination / limiting absorption / dilution:
  Following an **oral exposure**, consult a doctor/medical service. Do not induce vomiting or give anything by mouth to an unconscious person.
  Following a **dermal exposure**, the skin should be washed with copious amounts of tap water and soap. Contaminated clothing and footwear should be removed. Consult a doctor.
  Following **inhalation exposure**, leave the contaminated area immediately and move to fresh air. Consult a doctor.
  Following **ocular exposure**, remove any contact lens at once. Eyes should be immediately irrigated with copious amounts of water for at least 15 minutes. Irrigation should not be delayed for the purpose of obtaining sterile or special irrigating solutions. Consult a doctor.

B.6.9.6 Expected effects of poisoning

In the reported cases, there appeared to be a delayed type hypersensitivity reaction (type IV allergic reaction) including itching, skin rash, swollen eyes and face, burning and mucous membrane irritation. Symptoms typically develop over a few hours to several days following exposure. Affected individuals make a full recovery, with no long term adverse consequences, within a short period of time.

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ARfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of mammalian toxicology

Adsorption, distribution, metabolism, excretion

Metabolic and kinetic studies were conducted with radiolabeled fluazinam, following oral administration at a low dose of 0.5 mg/kg bw, a high dose of 50 mg/kg bw and 14 daily oral

doses of unlabeled fluazinam followed by ^{14}C-fluazinam (labelled in the phenyl position) of 0.5 mg/kg bw. The majority of radiolabeled material was detected in the feces (> 88 %). Urine was a minor excretory route (2 - 4 %). Less than 1 % of the administered dose was found in the carcass. The highest concentration was detected in the liver. There were no major differences related to sex or dose level. The median peak time for blood concentration of radiolabel activity for both sexes was 6 hours. At the time of peak concentration, the radioactivity in the blood represented 0.4 % - 0.6 % of the administered dose for 0.5 and 50 mg/kg bw dose groups. By 72 hours, about 0.1 % of the administered dose was found in the blood of both sexes at both dose levels. Approximately 30 % (high dose) – 40 % (low dose) of fluazinam was considered to be absorbed based on excretion rates in bile and urine. The predominant route of excretion of the absorbed dose was the bile, which contained approximately 87 % of the absorbed dose. 24 hours after dose administration, biliary excretion of the absorbed dose was 80 % complete at the high dose level and 92 % complete at the low dose level.

Metabolites were identified using several techniques including HPLC coelution with standards, direct identification by mass spectrometry and comparison with standards, NMR, and degradation experiments. The distribution of these metabolites, as a function of dosing regimen, position of radiolabel, and sex, was determined. Major metabolites isolated and identified from feces, urine and bile were the parent compound, DAPA, AMPA, AMPA mercapturate, DAPA glucuronide and DAPA cysteine conjugate. The major metabolites of the organic fraction of feces were parent compound, AMPA and DAPA and the major metabolite in the aqueous fraction of feces was DAPA cysteine conjugate. The feces were the major route of excretion of fluazinam and its metabolites. AMPA mercapturate, DAPA glucuronide and DAPA were found in the urine at low levels (≤ 2 % of administered dose) and AMPA mercapturate and DAPA glucuronide were found in the bile (≤ 5 % of administered dose). Fluazinam was also metabolized by the intestine microflora to form AMPA and DAPA. The identified metabolites were the same in samples from both phenyl and pyridyl labels, indicating that metabolic cleavage of the two rings did not occur. The metabolism of fluazinam was similar between male and female rats within a dose group. It can be concluded that fluazinam is metabolized by both reduction and glutathione conjugation and further metabolism.

Acute toxicity

After oral application to mice and rats of both sex, fluazinam is of low acute toxicity with LD_{50} values ≥ 4100 mg/kg bw.

After acute dermal application of fluazinam to rats of both sex, the acute dermal LD_{50} was > 2000 mg/kg bw. The inhalative LC_{50} of fluazinam in rats (whole-body exposure) was 0.46 mg/l.

Fluazinam is mildly irritating to the skin and severely irritating to the eyes of New Zealand White rabbits. In the Magnusson and Kligman dermal maximization study and in the Buehler-Test fluazinam caused evidence of delayed contact hypersensitivity in guinea pigs. A

summary of the results from the acute toxicity studies is presented in table 6.2.7-1. According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as toxic by inhalation, severely irritating to the eyes (risk of serious damage to eyes) and as a sensitizer (hazard symbols T, Xi, risk phrases R 23, 41, 43).

Table 6.10.1-1 Summarised results of the acute toxicity studies with fluazinam

Type of study	Species	Vehicle	Results	Reference
Acute oral toxicity	CD-1 mice	Maize oil	m/f > 5000 mg/kg bw	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley - Rat	Maize oil	m/f > 5000 mg/kg bw	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat	Methylcellulose	m 4500 mg/ kg bw f 4100 mg/ kg bw	*Liggett, 1988*
Acute dermal toxicity	Sprague Dawley Rat	-	m/f > 2000 mg/kg bw	*Cummins, 1988*
Acute inhalation toxicity	Sprague Dawley Rat	Polyethylen glycol 400	m 0.463mg/l air f 0.476 mg/l air (4h, whole body exposure)	*Tobeta, 1988*
Dermal irritation study	Rabbit (NZW)	Moistened with deionized water	Mildly irritating	*Shults, 1992*
Eye irritation study	Rabbit (NZW)	-	severely irritating	*Shults, 1992*
Dermal sensitization M & K-test	Guinea pig (Dunkin Hartley)	Paraffin oil	Sensitizing	*Cummins, 1984*
Dermal sensitization Buehler –test	Guinea pig (Dunkin Hartley)	Polysorbate 80	Sensitizing	*Pritchard, 1986*

Short term toxicity

Subacute and subchronic administration of fluazinam to rats, mice and dogs caused reduced food consumption and body weight gain. Changes of hematological parameters such as lower haemoglobin concentrations, lower erythrocyte counts and lower platelet counts were also observed. Clinical chemistry parameters showed low ALT activity, higher cholesterol, phospholipid and glucose concentrations. Higher absolute and relative liver weights and histopathological changes in the liver such as periacinar hepatocytic hypertrophy were observed in all species. In mice and dogs, vacuolation of white matter in brain and spinal cord was observed. High dosed dogs of the 4- and 13-week oral toxicity studies (150 and 100 mg/kg bw/d resp.) showed retinal hyperreflection and grey pigmentation of the tapetal fundus of the retina. At histopathologic examination, a dystrophy of the pigment epithelium of the retina was observed in the majority of dogs, including controls. The toxicological significance of the ophthalmic observations and the possible interrelationships between these and the retinal findings observed histopathologically were unknown. Oral administration of 200/150 mg/kg bw/d fluazinam to beagle dogs for 11 weeks revealed ERG-

abnormalities which can be accounted for by functional changes in the pigment epithelium of the retina. The results show recovery of response amplitude after withdrawal of fluazinam, but it is not possible to say if recovery would be complete.

Dermal administration of fluazinam to rats for 3 weeks revealed changes in clinical chemistry parameters such as higher AST activity and higher cholesterol levels in all dose groups (10, 100 and 1000 mg/kg bw). A toxic effect was also observed histopathologically in the liver in both sexes of the high dose and in males of the mid dose groups (periacinar hepatocytic hypertrophy). Dermatitis and acanthosis of the skin were seen in all dose groups compared to controls.

Table 6.10.1-2: Summarised results of subacute/subchronic toxicity studies with fluazinam

Study; Reference	Dose levels	NOAEL	Relevant effects
CD rats 4 weeks oral *Broadmeadow A. et al; 1983*	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.26, 5.21, 26.1 and 305.4 mg/kg bw)	1.26 mg/kg bw/d	-reduced food consumption and body weight gain -hematological and clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the liver
CD rats 13 weeks oral *Broadmeadow A. et al; 1985*	0 and 500 ppm/diet (equivalent to 0 and ≈ 40 mg/kg bw/d)	Cannot be determined	-reduced body weight gain -higher relative liver weights -histopathological changes in the liver
CD rats 13 weeks oral *Broadmeadow A. et al; 1984*	0, 2, 10, 50 and 500 ppm/diet (equivalent to 0, 0.16, 0.82, 4.1 and 41 mg/kg bw)	4.1 mg/kg bw/d	-hematological findings -higher relative liver weights -higher absolute and relative lung and uterus weights -histopathological changes in the liver
CD rats 21 days dermal *Cummins H. A. et al; 1985*	0, 10,100 and 1000 mg/kg bw)	Cannot be determined	-reduced body weight gain -clinical chemical findings -higher absolute and relative liver weights -encrustations or staining of the skin -histopathological changes in the liver and skin
CD-1 mice 4 weeks oral *Amyes S. J. et al; 1983*	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.6, 7.9, 39.5 and 455 mg/kg bw)	1.6 mg/kg bw/d	-reduced food consumption and body weight gain -clinical chemical findings -higher absolute and relative liver and kidney weights -histopathological changes in the liver
CD-1 mice 4 weeks oral *Chambers P. R. et al; 1994*	0, 3000, 5000 and 7000 ppm/diet (equivalent to 0, 607, 994 and 1302 mg/kg bw)	Cannot be determined	-clinical chemical findings -higher absolute and relative liver and kidney weights -histopathological changes in liver and kidneys -vacuolation of white matter in brain and spinal cord

Study; Reference	Dose levels	NOAEL	Relevant effects
CD-1 mice 13 weeks oral *Dawe S. et al; 1985*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.13, 1.23, 14.4 and 135.28 mg/kg bw for males and 0, 0.15, 1.58, 15.07 and 152.45 mg/kg bw for females)	Cannot be determined	-higher absolute and relative liver and kidney weights
Beagle dogs 4 weeks oral *Broadmeadow A. et al; 1984*	0, 1, 5, 25 and 150 mg/kg bw, gelatine capsules)	5 mg/kg bw/d	-reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -higher relative liver weights
Beagle dogs 13 weeks oral *Broadmeadow A. et al; 1985*	0, 1,10 and 100 mg/kg bw, gelatine capsules)	10mg/kg bw/d	-reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the liver
Beagle dogs 11 weeks oral *Hull R. M. et al; 1986*	0 and 200/150 mg/kg bw, gelatine capsules)	Not determined	-reduced food consumption and body weight gain -clinical chemical findings -brown granularity of the tapetal fundus of the retina -ERG-abnormalities
Beagle dogs 52 weeks oral *Broadmeadow A. et al; 1987*	0, 1, 10 and 50 mg/kg bw, gelatine capsules)	1mg/kg bw/d	-reduced food consumption and body weight gain -hematological and clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the stomach -vacuolation of white matter in brain and spinal cord

Genotoxicity

Mutagenicity assays performed with fluazinam *in vitro* included gene mutation tests in bacteria (*S. typhimurium and E.coli*) and in mammalian cells (*mouse lymphoma*), a chromosomal aberration test in mammalian cells (Chinese hamster lung fibroblasts) and a DNA repair test in bacteria (Bacillus subtilis). Results from these studies showed that fluazinam did not induce gene mutation in any of the bacterial tester strains of *S. typhimurium* and *E.coli,* or gene mutation in mammalian cells in culture (mouse lymphoma). No potential for clastogenicity was observed in the in vitro chromosome aberration test in chinese hamster lung fibroblasts (CHL). There was also no induction for DNA damage observed in the DNA repair test with B.subtilis.

In the *in vivo micronucleus test* no induction of micronuclei by fluazinam in mouse bone marrow cells could be observed (table 6.4.3 -1).

Table 6.10.1-3: Summarised results of genotoxicity studies with fluazinam

Type of study	Test system	Dose range	Results	Reference
In vitro-studies				
Bacterial mutation assay	S. typhimurium (TA1535, TA1537, TA98 and TA100) and E. coli WP2uvrA/pKM101 (CM891)	0.005, 0.015, 0.050, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate	Negative	*Kitching J.;2000*
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0625 - 2 µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 – 500 µg/plate (with S-9 mix)	Negative	*Ohtsuka M.; 1988*
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0313 - 1µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 - 500 µg/plate ((with S-9 mix)	Negative	*Ohtsuka M.; 1989*
Mammalian cell mutation assay	mouse lymphoma L5178Y cells	First test: 0.05 - 5 µg/ml (without S-9 mix); 0.5 - 20 µg/ml (with S-9 mix) Second test: 0.005 - 0.5 µg/ml (without S-9 mix); 0.5 – 10 µg/ml (with S-9 mix)	Negative	*Ransome S.; 2000*
Chromosomal aberration test	CHL	1 - 4 µg/ml (with S-9 mix); 2.375 - 9.5 µg/ml (without S-9 mix)	Negative	*Kajiwara Y.; 1988*
DNA repair test	bacillus subtilis	0.003 - 0.3 µg/disk (without S-9 mix), 0.3 – 30 µg/disk (with S-9 mix)	negative	*Ohtsuka M.; 1988*
In vivo-studies				
Micronucleus test	mouse bone marrow	single oral doses of 0, 500, 1000 and 2000 mg/kg bw	negative	*Matsumoto K.; 1999*

Long term toxicity/carcinogenicity

In the two long term toxicity/carcinogenicity studies in rats, treatment-related non-neoplastic effects were manifest at 100 ppm especially in the liver and testes. No treatment-related effects were seen on the spontaneous tumor profile at any dose level. Taking the two long term toxicity/carcinogenicity studies in rats together, an overall NOAEL for fluazinam can be obtained at 50 ppm, equivalent to 1.9 mg/kg bw/d for males and 2.4 mg/kg bw/d for females. In two carcinogenicity studies in mice, liver cell tumours (adenomas and carcinomas) were observed in a greater number of male mice after dietary administration of 1000, 3000 and 7000 ppm fluazinam, reaching statistical significance for adenomas at dose levels of 1000 (33 %) and 3000 ppm (40 %) only. The historical control data for liver tumours carried out at Huntingdon Research Centre Ltd. in the years 1981 – 1983 and 1991 – 1993 showed

incidences of adenomas in the range of 3.8 to 34 %. Thus the incidence of liver tumours at 1000 and 3000 ppm were within or slightly above the range of the historical control data. However, hepatocellular adenomas in the highest dose group of 7000 ppm reached an incidence of 28% and were within the range of the historical controls.

A statistically significant increase of vacuolation of white matter in the brain and cervical spinal cord was observed in both sexes at dose levels of 1000 ppm fluazinam and above. 10 ppm, equivalent to 1.12 mg/kg TG/d for males and 1.16 mg/kg TG/d for females, were considered to be the NOAEL in carcinogenicity studies in mice.

Table 6.10.1-4: Summarised results of long term toxicity studies with fluazinam

Study; Reference	Dose levels	NOAEL	Main effects/target organs
Sprague-Dawley rats 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.04, 0.38, 3.82 and 40 mg/kg bw males, 0, 0.05, 0.47, 4.87 and 53 mg/kg bw females)	10 ppm (0.38 mg/kg bw males, 0.47 mg/kg bw females)	-hematological and clinical chemical findings -higher liver and thyroid weights -histopathological changes in liver, pancreas, lungs and testes
Sprague-Dawley rats 104 weeks oral *Chambers P. R. et al; 1993*	0, 25, 50 and 100 ppm/diet (equivalent to 0, 1.0, 1.9 and 3.9 mg/kg bw males, 0, 1.2, 2.4 and 4.9 mg/kg bw females)	50 ppm (1.9 mg/kg bw males, 2.4 mg/kg bw females)	-higher liver, testes and epididymides weights -histopathological changes in liver, pancreas, lungs and testes
CD-1 mice 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.12, 1.12, 10.72 and 107 mg/kg bw males, 0, 0.11, 1.16, 11.72 and 117 mg/kg bw females)	10 ppm (1.12 mg/kg bw males, 1.16 mg/kg bw females)	-higher liver weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord
CD-1 mice 104 weeks oral *Chambers P. R. et al; 1998*	0, 1000, 3000 and 7000 ppm/diet (equivalent to 0, 126, 377 and 964 mg/kg bw males, 0, 162, 453 and 1185 mg/kg bw females)	Cannot be determined	-higher liver, brain and adrenal weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord

Reproductive toxicity:

In a two generation reproduction study, rats fed a diet containing fluazinam in the highest concentration of 500 ppm showed statistically significant reductions in body weight and body weight gain and reduced food intake. Relative liver weights were significantly increased in both sexes of the highest dose group and also in females of the intermediate and low dose group of the F0 generation, but a clear dose response was not observed. High dose males of the F1 generation showed also an increase of relative liver weight. Histopathologically, an statistically significant increase of periacinar hepatocytic fatty changes were detected in high dose males of F0 and F1 animals and also in F1 males of the 100 ppm group. The NOAEL for systemic toxicity was considered to be 20 ppm, equivalent to approximately 1 mg/kg bw/d for males and 1.4 mg/kg bw/d for females.

Reproductive performance of F0 animals was unaffected by treatment. In the F1 generation,

conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups. Numbers of implantation sites and mean litter sizes to day 4 post partum were slightly reduced for F1 animals of the high dose group and marginally lower in the intermediate group (100 ppm). The NOAEL for reproductive parameters was considered to be 20 ppm, equivalent to approximately 1 mg/kg bw/d for males and 1.4 mg/kg bw/d for females.

Two teratology studies in rabbits had been performed. In the first study, dose levels of 0.3, 1 and 3 mg/kg bw fluazinam from day 6 to 19 of gestation had been chosen. There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d). In the high dose group of 3 mg/kg bw fluazinam, reduced food intake and incomplete ossification were observed. Based on these results, the NOAEL for maternal toxicity and fetal toxicity was obtained at 1 mg/kg bw/d.

In the second study, oral administration of fluazinam to pregnant rabbits during the period of organogenesis was associated with reduced maternal weight gain and food intake in the highest dose group of 12 mg/kg bw/d. Macroscopic and microscopic lung and liver changes were observed at a dose level of 4 mg/kg bw/d and above. So the maternal NOAEL was considered at 2 mg/kg/day. Increased incidences of fetal abnormalities (placental abnormalities, some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were seen at the top dose. At all dose levels, increased incidences of preimplantation losses were observed, however, the values fell within the recorded background control range of the laboratory. Based on increased abortion and postimplantation loss from 4 mg/kg bw/d upwards, the NOAEL for developmental effects was set at 2 mg/kg bw/d.

Taking the two teratology studies in rabbits together, an overall NOAEL for maternal and fetal toxicity can be obtained at 2 mg/kg bw/d.

In a teratology study in rats, oral administration of fluazinam at the high dose level of 250 mg/kg bw/d to pregnant rats during the period of organogenesis was associated with reduced mean food consumption and weight loss, followed by a slight reduced rate of weight gain compared to controls. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. So the maternal NOAEL was considered at 10 mg/kg/day. Fetal and placental weights were significantly reduced in the high dose group and there were indications of fetal immaturity. In the 50 mg/kg bw/d group, fetal and placental weights were reduced, but not significantly, compared to controls. An increased incidence of gross morphological fetal abnormalities were recorded at the top dose, values were outside the range of the concurrent controls and the recorded background controls of the laboratory. So the NOAEL for developmental effects was considered at 10 mg/kg bw/d. According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam should be classified to "category 3 of reproductive substances" and labelled with the risk phrase "R 63 – Possible risk of harm to the unborn child".

Table 6.10.1-5: Summarised results of reproductive toxicity/teratogenicity studies with fluazinam

Study Reference	Dose levels	NOAEL	Main effects/target organs
Two generation reproduction, rats *Tesh J. M. et al; 1987*	0, 20, 100 or 500 ppm, equivalent to 0, 1, 5 and 26 mg/kg bw/d males; 0, 1.4, 6.7 and 34 mg/kg bw/d females (lowest value of the range)	Parental and Reproductive NOAEL 20 ppm (1 mg/kg bw/d males, 1.4 mg/kg bw/d females)	Parental: body weight and body weight gain ↓; relative liver weight ↑ Offsprings: gestation length ↑; implantation sites and litter sizes ↓
Teratology in the rabbit *Tesh J. M. et al; 1985*	0, 0.3, 1 and 3 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 1 mg/kg bw/d Developmental NOAEL 1 mg/kg bw/d	Maternal: food consumption ↓ Developmental: ossification incomplete
Teratology in the rabbit *Tesh J. M. et al; 1988*	0, 2, 4, 7 and 12 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 2 mg/kg bw/d Developmental NOAEL 2 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓; histopathological liver changes Developmental: abortion ↑; postimplantation loss ↑
Teratology in the rat *Willoughby C. R. et al; 1985*	0, 10, 50 and 250 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 10 mg/kg bw/d Developmental NOAEL 10 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓ Developmental: fetal and placental weight ↓; ossification incomplete; gross morphological fetal abnormalities

Neurotoxicity:

Single oral doses (gavage) of 1000 and 2000 mg/kg bw fluazinam produced statistically significantly lower motor activity in female rats compared to controls. No pathological findings were observed at gross necropsy examination and no histopathological findings were seen in the sections of nervous tissues examined. The NOAEL based on systemic toxicity was considered to be 50 mg/kg bw.

After 13 weeks of treatment with fluazinam in the diet, no evidence of neurotoxicity and neuropathology during the course of the study was observed. Reduced locomotor activity observed in males during week 8 of treatment compared to controls was not considered to

be treatment related as there were no statistically significant differences during week 13. The NOAEL for neurotoxicity was established at 1000 ppm (69 mg/kg bw). The NOAEL for systemic toxicity was established at 300 ppm (21 mg/kg bw/d), based on statistically significantly lower body weight gains among females treated with 1000 ppm fluazinam.

Further toxicological studies:

Metabolites:

The metabolite G-450, HYPA, chemical name 5-((3-chloro-5-(trifluoromethyl)-2-pyridyl)amino)- α,α,α- trifluoro-4,6-dinitro-o-cresol, detected in liver, kidney, muscle, fat and eggs of Laying hens but not in rat metabolism studies, was found to be more toxic than fluazinam after oral administration. The acute oral median lethal dose (LD_{50}) of HYPA in mice was 331 mg/kg bw (fluazinam is of low acute toxicity with LD50 values $\geq$ 4100mg/kg bw in mice and rats).

In an Ames- test, HYPA showed slight reverse mutagenicity against S. typhimurium TA98 without S-9 mix. The other bacteria tester strains (S. typhimurium TA100, TA1535, TA1537 and E.coli WP2uvrA) showed no increase in the number of revertant colonies at any dose level. In a second Ames-test, carried out with the same tester strains, HYPA showed no evidence of mutagenic activity, either in the presence or absence of metabolic activation. Micronucleus tests showed that HYPA does not induce micronuclei in the bone marrow cells of male and female mice.

Metabolite G-525, MAPA, chemical name 2-(2-amino-3-chloro-α,α,α-trifluoro-6-nitro-*p*-toluidino)-3-chloro-5-(trifluoromethyl)pyridine was found in liver, kidney, muscle, fat and eggs of Laying hens but not in rat metabolism studies and showed only low acute toxicity with a LD_{50} > 5000 mg/kg bw in mice.

In a bacterial reverse mutation test, MAPA showed no increase in the number of revertant colonies at any dose level.

Impurities:

Impurity G-624, chemical name [redacted] showed low acute oral toxicity in rats of both sexes with a median lethal dose (LD_{50}) of > 5000 mg/kg bw. In a bacterial reverse mutation test, G-624 showed reverse mutagenicity against S. typhimurium TA98, TA100, TA1535 and TA1537 with and without S-9 mix and also against *E. coli* (WP2 uvr A) in the absence of S-9 mix. However, G-624 is included in toxicity studies with the active ingredient. There was no potential of genotoxicity seen with fluazinam technical, so no further studies with G-624 are required.

Dietary administration of Impurity-5, chemical name [redacted], to male mice at a concentration of 5 mg/kg bw caused clinical signs, decreased body weights, increased brain weights, edema of the brain and vacuolation of white matter in the brain. The oral administration of other 8 impurities at a single oral dose of 50 mg/kg bw or higher caused no adverse effects on mortality, clinical signs, body and brain weight, macroscopic pathology or histopathology of the brain.

The neurotoxic effect of Impurity 5 in mice and rats was comparable in quality and strength. In dogs, the magnitude and extent were only trace and focal. The lower susceptibility in dogs might be imputed to that further older dogs were not utilized. Adult mice and rats are more sensitive to neurotoxicity of Impurity 5 than pubescent ones. The two-divided administration method used for dogs to prevent vomiting might be another reason for the minor appearance of the brain histopathological alteration in dogs.

Based on the results obtained in the study, the effects of Impurity-5 on the brain and optic nerves of mice were considered to increase with animal age until about ten weeks and then remain at a constant level until at least 24 weeks.

After 14 days of treatment with Impurity-5, female rats and mice showed similar sensitivity to the vacuolation of the white matter of the brain.

The incidence and severity of white matter vacuolation of the brain is similar in male mice and rats, but ten-week old animals were more sensitive in both rats and mice as compared to the three-week old animals.

General Pharmacology:

At intraperitoneal doses of 80 mg/kg bw fluazinam technical and less, there were no changes in the condition of male or female mice. Though no death was caused in the groups of 160 mg/kg bw, a descent of body temperature was seen. Mice of the highest dose group (320 mg/kg bw) died without showing special symptoms expect the descent of body temperature.

After administration of 0.5 mg/kg bw fluazinam i.v., a decrease of the number of pulse was observed. There were no effects on brain waves following i.v. administration of 0.5 mg/kg bw fluazinam. Following administration of 1.0 mg/kg bw, both cortical and deep brain waves declined but recovery was seen after 30 minutes. A dose dependent temporary rise in blood pressure and a decrease in pulse were observed in rabbits at a dose of 1.0 mg/kg bw fluazinam i.v., with recovery after 30 minutes. No change in the electrocardiogram and no effect on the pupils nor the contraction of tibialis anterior muscle of rabbits was observed.

Suppression in carbon powder transportability in the small intestine was observed following subcutaneous administration of a high dose of 5000 mg/kg bw fluazinam. Doses of 2500 mg/kg and less caused no observable changes. Slight hemolysis was observed in erythrocytes of rabbits by adding fluazinam at a concentration of 1 mg/ml.

The reversibility of vacuolation of white matter in the brain was tested after dietary administration of fluazinam at high concentrations (714 and 1743 mg/kg bw in rats; 1043, 1173 and 1871 mg/kg bw mice). The affected animals recovered nearly completely after a 25 day or 56 day recovery period respectively, indicating that the histopathological change is reversible.

Representative electron-microscopic photographs of the cerebellum white matter indicated that the effect of treatment appeared to be confined to the myelin sheaths, that the nucleus and mitochondria in oligodendroglia were kept intact and that myelin sheaths had recovered during the recovery period.

In studies in which high doses of Fluazinam technical failed to induce white matter

vacuolation in the CNS, the batch contained very low levels of Impurity-5 (< 0.005 %).The threshold dose was independent of the Impurity-5 level in fluazinam technical. The CNS effect depends on the dose of Impurity-5 received by experimental animals. No white matter vacuolation was observed in rats, mice and dogs at dose levels of Impurity-5 below approximately 0.1 mg/kg bw/d.

Medical data:

No reports on exposure of general population and epidemiological studies have been submitted.

In the reported cases on manufacturing plant personnel and farmers, there appeared to be a delayed type hypersensitivity reaction (type IV allergic reaction) including itching, skin rash, swollen eyes and face, burning and mucous membrane irritation. Symptoms typically develop over a few hours to several days following exposure. Affected individuals make a full recovery, with no long term adverse consequences, within a short period of time.

Table 6.10.1-6: Summary of repeat oral dose studies with fluazinam for setting the ADI/AOEL/ARfD

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
CD rats 4 weeks oral Broadmeadow A. et al; 1983	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.26, 5.21, 26.1 and 305.4 mg/kg bw)	10 ppm (1.26 mg/kg bw/d)	50 ppm (5.21 mg/kg bw/d)	-higher relative liver weights
CD rats 13 weeks oral Broadmeadow A. et al; 1984	0, 2, 10, 50 and 500 ppm/diet (equivalent to 0, 0.16, 0.82, 4.1 and 41 mg/kg bw)	50 ppm (4.1 mg/kg bw/d)	500 ppm (41 mg/kg bw/d)	-hematological findings -higher relative liver weights -higher absolute and relative lung and uterus weights -histopathological changes in the liver
CD-1 mice 4 weeks oral Amyes S. J. et al; 1983	0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.6, 7.9, 39.5 and 455 mg/kg bw)	10 ppm (1.6 mg/kg bw/d)	50 ppm (7.9 mg/kg bw/d)	-clinical chemical findings
Beagle dogs 4 weeks oral Broadmeadow A. et al; 1984	0, 1, 5, 25 and 150 mg/kg bw, gelatine capsules)	5 mg/kg bw/d	25 mg/kg bw/d	-higher relative liver weights

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
Beagle dogs 13 weeks oral Broadmeadow A. et al; 1985	0, 1,10 and 100 mg/kg bw, gelatine capsules)	10mg/kg bw/d	100 mg/kg bw	-reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -clinical chemical findings -higher absolute and relative liver weights -histopathological changes in the liver
Beagle dogs 52 weeks oral Broadmeadow A. et al; 1987	0, 1, 10 and 50 mg/kg bw, gelatine capsules)	1mg/kg bw/d	10 mg/kg bw	-hematological findings -higher absolute and relative liver weights -histopathological changes in the stomach
Sprague-Dawley rats 104 weeks oral Chambers P. R. et al; 1993	0, 25, 50 and 100 ppm/diet (equivalent to 0, 1.0, 1.9 and 3.9 mg/kg bw males, 0, 1.2, 2.4 and 4.9 mg/kg bw females)	50 ppm (1.9 mg/kg bw males, 2.4 mg/kg bw females)	100 ppm (3.9 mg/kg bw males, 4.9 mg/kg bw females)	-higher liver, testes and epididymides weights -histopathological changes in liver, pancreas, lungs and testes
CD-1 mice 104 weeks oral Mayfield R. et al; 1988	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.12, 1.12, 10.72 and 107 mg/kg bw males, 0, 0.11, 1.16, 11.72 and 117 mg/kg bw females)	10 ppm (1.12 mg/kg bw males, 1.16 mg/kg bw females)	100 ppm (10.72 mg/kg bw males, 11.72 mg/kg bw females)	-higher liver weights -histopathological changes in liver
Two generation reproduction, rats Tesh J. M. et al; 1987	0, 20, 100 or 500 ppm, equivalent to 0, 1, 5 and 26 mg/kg bw/d males; 0, 1.4, 6.7 and 34 mg/kg bw/d females (lowest value of the range)	Parental and Reproductive NOAEL 20 ppm (1 mg/kg bw/d males, 1.4 mg/kg bw/d females)	100 ppm (5 mg/kg bw/d males, 6.7 mg/kg bw/d females)	Parental: relative liver weight ↑ Offsprings: gestation length ↑; implantation sites and litter sizes↓
Teratology in the rabbit Tesh J. M. et al; 1985	0, 0.3, 1 and 3 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 1 mg/kg bw/d Developmental NOAEL 1 mg/kg bw/d	Maternal LOAEL 3 mg/kg bw Developmental LOAEL 3 mg/kg bw/d	Maternal: food consumption ↓ Developmental: ossification incomplete
Teratology in the rabbit Tesh J. M. et al; 1988	0, 2, 4, 7 and 12 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 2 mg/kg bw/d Developmental NOAEL 2 mg/kg bw/d	Maternal NOAEL 4 mg/kg bw/d Developmental NOAEL 4 mg/kg bw/d	Maternal: histopathological liver changes Developmental: abortion ↑; postimplantation loss ↑
Teratology in the rat Willoughby C. R. et al; 1985	0, 10, 50 and 250 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 10 mg/kg bw/d Developmental NOAEL 10 mg/kg bw/d	Maternal LOAEL 50 mg/kg bw/d Developmental LOAEL 50 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓ Developmental: fetal and placental weight ↓

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
CD rats Acute neurotoxicity Serrone D. M. 1995	0, 50, 1000 and 2000 mg/kg bw (oral application by gavage)	Systemic toxicity: 50 mg/kg bw Neurotoxicity: 2000 mg/kg bw	1000 mg/kg bw	-soft stool -lower motor activity
CD rats 13 weeks neurotoxicity Hughes E. W. 1998	0, 300 and 1000 ppm/diet (equivalent to 0, 21 and 69 mg/kg bw)	Systemic toxicity: 300 ppm (21 mg/kg bw) Neurotoxicity: 1000 ppm (69 mg/kg bw)	1000 ppm (69 mg/kg bw/d)	-reduced body weight gains

B.6.10.2 Proposal for ADI

The estimation of the Acceptable Daily Intake (ADI) is based on the lowest no-observed adverse effect level (NOAEL) observed in subchronic and chronic toxicity, carcinogenicity, reproduction and neurotoxicity studies with fluazinam. Given the results from all relevant studies (see table 6.10.1-6), the lowest NOAELs of 1.12 mg/kg bw/d and 1.0 mg/kg bw/d respectively were found in the 104-week chronic toxicity/cancerogenicity study in mice, the subchronic toxicity study (52 weeks) in dogs and the two generation study in rats. It can be concluded that fluazinam exhibits no mutagenic or oncogenic potential. In the rat teratology study, an increased incidence of gross morphological fetal abnormalities was recorded at the top dose of 250 mg/kg bw/d, values were outside the range of the concurrent controls and the recorded background controls of the laboratory. 10 mg/kg bw/d was considered as a clear NOAEL for developmental effects. Applying the standard uncertainty factor of 100 to account for intraspecies and interspecies variability to the NOAELs of 1.12 mg/kg bw/d and 1.0 mg/kg bw/d respectively from the mouse, rat and dog studies mentioned, results in an ADI of 0.01 mg/kg bw/d. This provided a 1000 fold safety factor over the NOAEL of 10 mg/kg bw/d in the teratology study in rats.

Remark: The notifier proposed an ADI of 0.01 mg/kg bw/d based upon the NOAEL of 1 mg/kg bw/d established in the dog subchronic toxicity study also.

B.6.10.3 Proposed ARfD

For the determination of the Acute Reference Dose (ARfD), results from oral studies that used acute or short term exposure are considered to be the most relevant. Fluazinam is of low acute oral toxicity but there was evidence of teratogenicity seen in a rat developmental study at the high dose level of 250 mg/kg bw/d.

The most appropriate study for selection of an ARfD seems to be the teratogenicity study in rabbits, with a NOAEL of 2 mg/kg bw/d for maternal and developmental effects. The standard uncertainty factor of 100-fold is applied to account for intraspecies and interspecies variability.

So an ARfD of 0.02 mg/kg bw/day is proposed for fluazinam.

Remark: The notifier proposed no ARfD due to the low acute oral toxicity of fluazinam.

B.6.10.4 Proposal for drinking water limit

The determination of a maximum allowable concentration (MAC) value in drinking water is not necessary, because according to Directive 91/414/EC only the ADI, ARfD and AOEL values have to be determined. According to Directive 98/83/EC a drinking water limit of 0.1 µg fluazinam/l is established.

B.6.10.5 Proposal for AOEL

According to the principles of Annex VI to Directive 91/414 EEC, the proposed acceptable operator exposure level should be established on the basis of the highest dose at which no adverse effect is observed in relevant studies in the most sensitive species. The setting of an AOEL is usually based on mid-term studies (i.e. subacute/ subchronic and reproduction or developmental toxicity studies) since these studies in most cases can be considered a more appropriate model for the actual operator exposure to be expected.

The lowest NOAEL of all relevant studies were found in the 52-week dog study, which is considered a mid-term study. This NOAEL of 1 mg/kg bw/d was based on haematological findings, higher absolute and relative liver weights and histopathological changes in the stomach at the next higher dose level of 10 mg/kg bw/d. This NOAEL was also supported by the NOAELs of 1.6 mg/kg bw/d and 1.2 mg/kg bw in the 4-week and 104-week toxicity study in mice and the NOAEL of 1.0 mg/kg bw/d in the 2-generation study in rats, respectively.

Using an absorption of 35 % (between 30 – 40 % of an oral dose of fluazinam is absorbed), the systemic equivalent of the 1 mg/kg bw/d NOAEL would be approximately 0.35 mg/kg bw/d. If a 100 fold safety factor is applied to the systemic NOAEL, an AOEL of 0.0035 mg/kg bw/d is established. Therefore a systemic AOEL of 0.0035 mg/kg bw/d is proposed.

Remark: The notifier proposed an systemic AOEL of 0.014 mg/kg bw/d based on the NOAEL of 4.1 mg/kg bw/d derived in the 13-week feeding study in rats.

B.6.11 Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)

Acute toxicity studies have been provided for a 50 % suspension formulation of different batch numbers (Codes: BXD2110/47/2; K15-6002; 0/0001014; RS 399/H), containing 500 g fluazinam/l.

B.6.11.1 Acute oral toxicity study

B-1216: Acute oral and dermal toxicities of a 50 % suspension formulation to the rat:

Reference.: *Barber J. E.; 1985;* Report No. CTL/P/1226

No test-guideline is mentioned in the study but it is in compliance with GLP principles. The study is considered acceptable.

Material and method:

5 male and 5 female SPF albino rats (source:Alderley Park) with a mean weight of 329 - 375

g (♂) and 199 – 233 g (♀) received a single dose of 2000 mg/kg bw formulation as supplied (Ref. No. BXD2110/47/2; 500 g a.i./l) by oral gavage. After administration all animals were kept under observation for further 15 days. Animals were checked for clinical signs and mortality daily. Body weights were recorded on the day before dosing, the day of dosing and on days 3, 4, 8 and 15. At termination all rats were necropsied and a macroscopic examination was performed.

Findings:

Clinical signs and mortality: No mortalities occurred. During the observation period, signs of systemic toxicity were seen in all animals within 6 hours after dosing and included diarrhoea, piloerection, staining around mouth and nose, urinary incontinence and upward curvature of the spine. All animals had recovered by day 6. Initially, a decrease in body weight was observed but by day 8 all of the body weights were increased when compared to the initial (day 1).

Pathology: No gross pathological findings were seen in the animals sacrificed at termination of the study. Due to the absence of macroscopic lesions, no samples were taken for histological examination.

Conclusion:

BXD2110/47/2 formulation is of low acute toxicity in rats after oral administration. The LD_{50} was calculated to be > 2000 mg/kg bw in males and in females.

B.6.11.2 Acute dermal toxicity study

B-1216: Acute oral and dermal toxicities of a 50 % suspension formulation to the rat:

Reference.: *Barber J. E.; 1985;* Report No. CTL/P/1226

No test-guideline is mentioned in the study but it is in compliance with GLP principles. The study is considered acceptable.

Material and method:

The formulation (Ref. No. BXD2110/47/2; 500 g a.i./l) was administered to the skin of the clipped dorsal area of 5 male and 5 female SPF albino rats (source:Alderley Park) with a mean weight of 329 - 375 g (♂) and 199 – 233 g (♀) at a single dose of 2000 mg/kg bw (as supplied) under semi-occlusive dressing for 24 hours. After the 24-hour exposure period, the dressing was removed and the skin cleansed free of any residual formulation. After administration all animals were kept under observation for further 15 days. Animals were checked for clinical signs, skin irritation and mortality daily. Body weights were recorded on the day of dosing and on days 3, 4, 8 and 15. At termination all rats were necropsied and a macroscopic examination was performed.

Findings:

Clinical signs and mortality: No mortalities occurred. During the observation period, signs of systemic toxicity were seen in all females and in four of the males 24 hours after dosing and included diarrhoea, chromodacryorrhoea, staining around mouth and nose, urinary incontinence and upward curvature of the spine. All animals had recovered by day 6. Signs

of skin irritation including desquamation, erythema, scabbing and thickening were observed in all animals. With the exception of one male and one female, all animals had recovered by the end of the study. Initially, a decrease in body weight was observed but by day 8 all of the body weights were increased when compared to the initial (day 1).

Pathology: No gross pathological findings were seen in the animals sacrificed at termination of the study. Due to the absence of macroscopic lesions, no samples were taken for histological examination.

Conclusion:

BXD2110/47/2 formulation is of low acute toxicity in rats after dermal administration with the LD_{50} being higher than 2000 mg/kg bw in males and in females.

IKF-1216 50 % SC: Acute percutaneous toxicity study in the rat:

Reference.: *Cummins H. A.; 1990;* Report No. 90/ISK156/0990

The study was conducted according to US EPA FIFRA Guideline Subdivision F, No. 81-2 and performed in compliance with GLP principles. The study is scientific valid and therefore acceptable.

Material and method:

Groups of 5 rats/sex (strain: Sprague-Dawley (CD); source: Charles River (U.K.) Limited) received a single dose of 2000 mg/kg bw IKF-1216 50 % SC formulation (batch no.K15-6002). The dose was applied as a thin layer covering the shaven dorsum and was covered by an unmedicated gauze dressing and aluminium foil. The dressings were removed 24 hours after administration. The dermal site was brushed free of the test material and wiped with wet disposable towels. Each test site was inspected daily for dermal reactions to treatment. Bodyweights were recorded on the day before dosing and on days 1, 8 and 15. The study was terminated on day 15. All animals were necropsied and macroscopic organ changes reported.

Findings:

There were no deaths and no systemic reaction to treatment. Local irritations at the site of application were erythema, exfoliation, sensitivity to touch, loss of flexibility and eschar formation. All animals achieved expected bodyweight gains during the 14-day observation period. Necropsy on day 15 revealed yellow staining at the application sites of 4 females.

Conclusion:

Under the conditions of this study, the acute percutaneous LD_{50} of IKF-1216 50 % SC formulation in rats of both sexes observed for a period of 15 days was greater than 2000 mg/kg bw.

B.6.11.3 Acute inhalation toxicity study

IKF-1216 50 % SC: Acute inhalation toxicity study in the rat:

Reference: *Cracknell, S.; 1991;* Report No. 91/ISK161/0270

Guideline: The study was conducted according to OECD Guidelines for Testing of Chemicals

(1981), Japanese MAFF Test Guidelines for Toxicology Studies (NohSan No. 4200, 59) and U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-3 (1984). The study is considered acceptable.

GLP: yes

Material and Methods:

Groups of 5 rats/sex (strain: Sprague-Dawley (SPF); source: Charles River U.K.) weighing between 164 and 178 g (males, 6 weeks old) and 204 and 216 g (females, 11 weeks old) were exposed for four hours (snout only exposure) to an atmosphere containing the maximum practicable concentration (0.55 mg/l) of the test formulation (IKF-1216 50 % SC). The targeted air concentration was 12.22 mg/l (nominal concentration). The poor efficiency of IKF-1216 50 % SC generation was attributed to the high viscosity of the test substance formulation. The test substance was diluted with distilled water (75% v/v) before use based on trials which established that a 75% dilution in distilled water was suitable for consistent atmosphere generation.

Animals were exposed in a stainless steel inhalation chamber. The mass median aerodynamic diameter (MMAD) of the aerosol particles ranged from < 2.0 to > 5.5 µm. Animals were observed for clinical signs during exposure and at 30 minute intervals during the first 3 hours after exposure and then twice daily until completion of 14 days of observation. Body weights were recorded daily until the end of the observation period. At the end of the 14-day observation period, all surviving rats were exanguinated and necropsied.

Findings:

Clinical signs and mortality: There were no deaths during the study. General observations of treated animals during the three hours following exposure included a decreased respiratory rate and shallow respiration, rales, moist fur, orange staining around the head, pigmented staining of the snout and pigmented orbital secretion. Signs that persisted in some rats, or that developed during the observation period, included irregular respiration, decreased respiratory rate and shallow respiration, rales, salivation, decreased motor activity, poorly groomed fur, piloerection, orange staining around the head, pigmented staining of the snout and pigmented orbital secretion. All animals were normal in appearance and behavior from Day 10 of the observation period.

Pathology: No abnormal findings were noted in any of the animals at necropsy.

Conclusion:

Based on study results, the median lethal concentration (LC_{50}) via inhalation was greater than the maximum achievable concentration of the formulation, 0.55 mg/l/4 h.

IKF-1216 500 SC: Acute inhalation toxicity study in rats:

Reference: *Ebino, K.;2001;* Report No. IET 00-0048

Guideline: The study was conducted according to OECD Guidelines for Testing of Chemicals (1981), Japanese MAFF Test Guidelines for Toxicology Studies (NohSan No. 4200, 59) and U.S. EPA OPPTS 870.1300 (1998). The study is considered acceptable.

GLP: yes

Material and Methods:

Fluazinam IKF-1216 500SC (Batch No. 0/0001014, 39.89%) was used in this study to evaluate the acute inhalation toxicity in two groups of five male and five female Sprague-Dawley (Crj:CD(SD)IGS) rats (source: Charles River Japan), weighing between 326 and 407 g (males) and 222 and 250 g (females). In consideration of the high viscosity of IKF-1216 500SC, a 5-fold dilution (distilled water) of the formulation was used for one group and the non-diluted formulation was used for the other group. Animals were exposed to the test substance mist for a four-hour period in a stainless steel whole body exposure chamber. Mean actual atmospheric concentration was 4.9 mg/l for the 5-fold dilution (corresponding to 0.98 mg/l of nondiluted IKF-1216 500 SC) and 1.15 mg/l for the non-dilution group. The ratios of the mean actual atmospheric concentration to the nominal concentration were 11.8 % (5-fold dilution group) and 6.8 % (non-dilution group). The mass median aerodynamic diameter (MMAD) of the aerosol particles were 5.0 μm and 1.8 μm (5-fold dilution group) and 5.5 μm and 1.6 μm (non-dilution group). Animals were observed at 2 hours during the exposure and at 0, 2 and 4 hours immediately following the 4-hour exposure and then once daily for 14 days.

Actual atmospheric concentrations and the particle size distributions of the test substance were measured at 1, 2 and 3 hours after initiation of the exposure.

Findings:

Clinical signs and mortality: There were no deaths during the study. Animals of the 5-fold dilution group could not be observed for clinical signs during the study due to a foggy mist in the chamber during exposure. No adverse observations were found in any animals in the non-dilution group at 2 hours after initiation of exposure.

Bradypnoea was observed in 2 males of the 5-fold dilution group and 1 male of the non-dilution group immediately after termination of the exposure. A red adhesive substance was observed in the nasorostral and mandibular regions of the animals in both groups and in the periocular region of animals in the non-dilution group. All clinical signs disappeared by day 3 in the 5-fold dilution group and by day 4 in the non-dilution group. One female of the non-dilution group showed hair loss on the face and lumbo-dorsal region from day 7 to day 12. One female of the 5-fold dilution group, one male and one female in the non-dilution group showed body weight loss (1 gram each) on day 7, but all animals showed favorable body weight gains by day 14.

Pathology: No abnormalities were observed in any animal at necropsy.

Conclusion:

Based on study results, the median lethal concentration (LC_{50}) via inhalation was greater than the maximum achievable concentration of the formulation, 1.15 mg/l/4 h.

B.6.11.4 Skin irritation

IKF-1216 50 % SC: Primary dermal irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK157/1280

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-5. The study is considered acceptable.

GLP: yes

Material and Methods:

The back of 6 male New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing between 2100 and 2770 g) was clipped free of hair with electric clippers. Two test sites (6 x 6 cm) were marked on either side of the clipped area. Each rabbit received 0.5 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water) on the left test site, the right site remained untreated, acting as control. Both sites were dressed with an occlusive wrap for an exposure period of 4 hours. Following the exposure period, the test sites were wiped with paper towels (wetted with water) and examined for local skin reactions and scored and evaluated for erythema, eschar and edema using the method of Draize (1959). Reading of the individual scores is reported within 60 minutes and then at approximately 24, 48 and 72 hours following removal of the patch and on days 7 and 10 of the study.

Findings:

Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study.

Slight to well defined erythema and slight oedema were observed in all 6 rabbits during the first 72 hours following bandage removal. On day 7, one rabbit displayed very slight erythema. The test sites of all animals were overtly normal on day 10. The mean values for erythema and oedema recorded 24, 48 and 72 hours after treatment did not equal or exceed the EEC limit values considered to indicate a significant inflammatory response to treatment, i.e. in this study, all scores were less than 2.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation was classified “non-irritant” to skin.

IKF-1216 50 % SC (1:500 dilution): Primary dermal irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK173/1282

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-5. The study is considered acceptable.

GLP: yes

Material and Methods:

The back of 6 male New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing between 2060 and 2720 g) was clipped free of hair with electric clippers. Two test sites (6 x 6 cm) were marked on either side of the clipped area. Each

rabbit received 0.5 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water) diluted 1:500 v/v in distilled water, on the left test site, the right site remained untreated, acting as control. Both sites were dressed with an occlusive wrap for an exposure period of 4 hours. Following the exposure period, the test sites were wiped with paper towels (wetted with water) and examined for local skin reactions and scored and evaluated for erythema, eschar and edema using the method of Draize (1959). Reading of the individual scores is reported within 60 minutes and then at approximately 24, 48 and 72 hours following removal of the patch.

Findings:

Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study.

There was no reaction to treatment at the test site of any animal at any time during the observation period.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation, diluted 1:500 v/v in distilled water, was classified “non-irritant” to skin.

IKF-1216 50 % SC (1:1000 dilution): Primary dermal irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK175/1284

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-5. The study is considered acceptable.

GLP: yes

Material and Methods:

The back of 6 male New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing between 2210 and 2830 g) was clipped free of hair with electric clippers. Two test sites (6 x 6 cm) were marked on either side of the clipped area. Each rabbit received 0.5 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water) diluted 1:1000 v/v in distilled water, on the left test site, the right site remained untreated, acting as control. Both sites were dressed with an occlusive wrap for an exposure period of 4 hours. Following the exposure period, the test sites were wiped with paper towels (wetted with water) and examined for local skin reactions and scored and evaluated for erythema, eschar and edema using the method of Draize (1959). Reading of the individual scores is reported within 60 minutes and then at approximately 24, 48 and 72 hours following removal of the patch.

Findings:

Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study.

There was no reaction to treatment at the test site of any animal at any time during the observation period.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation, diluted 1:1000 v/v in distilled water, was classified “non-irritant” to skin.

B -1216 50 % suspension formulation: Skin irritation and eye irritation studies:

Reference: *Southwood, J.; 1985;* Report No. CTL/P/1238

No test-guideline is mentioned in the study but it is in compliance with GLP principles. The study is considered acceptable.

Material and Methods:

The left flank of 6 male New Zealand White rabbits (source: Hacking and Churchill, Huntingdon, UK and Mellor Rabbits, Chadderton, UK, weighing between 2372 and 2616 g) was clipped free of hair (70 x 130 mm) with electric clippers. Each rabbit received 0.5 ml B -1216 50 % suspension formulation (Ref. No. JF9550, batch no. BXD2110/47/2; 500 g a.i./l) on the left flank, the site was dressed with an occlusive wrap for an exposure period of 4 hours. Following the exposure period, the test site was wiped with paper towels (wetted with water) and examined for local skin reactions and scored and evaluated for erythema, eschar and edema using the method of Draize (1959). Reading of the individual scores is reported 1, 21, 44, 68, 167 and 284 hours following removal of the patch.

Findings:

Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study.

Test sites were stained by the formulation an hour after treatment. In two animals staining was to such a degree that it would have obscured moderate erythema had it been present and persisted to prevent assessment of erythema up to 167 hours following decontamination in one rabbit and up to 68 hours in the other rabbit. At 21 hours, all 4 readable sites had well defined erythema, which regressed to very slight erythema by 44 hours and only persisted in one rabbit up to 68 hours. All signs of erythema had disappeared by 167 hours.

Very slight or well defined edema was seen in two rabbits one hour after decontamination and developed into severe edema in one rabbit and remained as very slight to well defined edema in four rabbits by 21 hours. All edema disappeared by 68 hours after decontamination. Slight desquamation was observed in two rabbits.

The mean values for erythema and edema were calculated to be 1.0 and 0.7, respectively.

Conclusion:

Under the conditions of this test, B -1216 50 % suspension formulation was classified “non-irritant” to skin.

B.6.11.5 Eye irritation

B -1216 50 % suspension formulation: Skin irritation and eye irritation studies:

Reference: *Southwood, J.; 1985;* Report No. CTL/P/1238

No test-guideline is mentioned in the study but it is in compliance with GLP principles. The

study is considered acceptable.

Material and Methods:

A single dose of 0.1 ml B -1216 50 % suspension formulation (Ref. No. JF9550, batch no. BXD2110/47/2; 500 g a.i./l) was administered to the left eye of nine female New Zealand White rabbits (source: Hacking and Churchill, Huntingdon, UK and Mellor Rabbits, Chadderton, UK, weighing between 2411 and 3019 g). The right eye remained untreated (control eye). Immediately after instillation of the formulation, an assessment of the initial pain reaction of each rabbit was made. Twenty to 30 seconds after instillation of the formulation, the eyes of three of the rabbits were irrigated for approximately one minute with approximately 200 ml of clean lukewarm water. The eyes were examined and the Draize scale was used to assess the grade of ocular reaction 1-2 hours, 1, 2, 3, 4 and 7 days after instillation of the formulation. In addition, fluorescein staining was used at 1, 2, 3, 4 and 7 days to aid in the assessment of corneal damage.

Findings:

Initial pain: One rabbit showed practically no initial pain (Class 1) and the remaining eight animals showed slight initial pain (Class 2), i.e. the rabbits blinked and tried to open the eye but the reflexes closed it.

Irrigated eyes: One to two hours following instillation, all three animals had slight or moderate conjunctival redness (score 1 – 2) and slight or moderate chemosis (score 1 – 2). Slight discharge (score 1) was seen in two animals only. All animals had recovered 7days following instillation. The only other observation was test substance in the conjunctival sac.

Non-irrigated eyes: One to two hours following instillation, all six animals had slight or moderate conjunctival redness, slight or moderate chemosis and slight or moderate discharge. All animals recovered 7 days following instillation. The only other observations were Harderian discharge and test substance in the conjunctival sac.

Conclusion:

Under the conditions of this test, B -1216 50 % suspension formulation was a mild irritant to both the irrigated and non-irrigated rabbit eye.

IKF-1216 50 % SC: Primary eye irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK158/1281

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-4. Because one animal was used for the test only, it is an invalid study.

GLP: yes

Material and Methods:

One male New Zealand White rabbit (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing 2650 g) received 0.1 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water), instilled into the right eye. The left eye remained untreated (control eye). Immediately after instillation of the formulation, an assessment of the

initial pain reaction of the rabbit was made. The eyes were examined and assessed at least twice during the first hour and at regular intervals throughout the day to ensure no severe injury passed un-noticed. Ocular reactions were assessed one hour and 24 hours only after instillation. The animal was checked daily to ensure the treated eye was not subject to infection or causing distress.

Findings:

Instillation of the test material caused a very slight initial pain response. Injection of the conjunctival blood vessels and very slight chemosis were observed in the single animal one hour after instillation of the test material. At 24 hours, a diffuse beefy-red appearance to the conjunctiva, slight chemosis, moderate discharge and iritis were observed. These changes were accompanied by several areas of hemorrhage on the inside of the upper eye lid and the nictitating membrane. The animal was killed immediately following this examination. Because of the severe reaction observed in this sentinel animal, no further animals were used on the study.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation was “severely irritant” to the rabbit eye, but due to the fact that only one animal was used, a conclusion is not possible.

IKF-1216 50 % SC (1:500 dilution): Primary eye irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK174/1283

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-4. The study is considered acceptable.

GLP: yes

Material and Methods:

Six male New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing 2440 - 2830 g) received 0.1 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water), diluted 1:500 v/v in distilled water, instilled into the right eye. The left eye remained untreated (control eye). Immediately after instillation of the formulation, an assessment of the initial pain reaction of the rabbits was made. The eyes were examined and assessed at least twice during the first hour and at regular intervals throughout the day to ensure no severe injury passed un-noticed. Ocular reactions to treatment were assessed one hour, 24, 48 and 72 hours after instillation. The animals were checked daily to ensure the treated eyes were not subject to infection or causing distress.

Findings:

Instillation of the test material caused a very slight initial pain response in one animal. The other 5 rabbits showed no reaction. Injection of the conjunctival blood vessels was observed in one animal at the one hour examination and in a second animal at the 24 and 48 hour examinations. No other ocular reaction was observed.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation, diluted 1:500 v/v in distilled

water, was classified “non-irritant” to the rabbit eye.

IKF-1216 50 % SC (1:1000 dilution): Primary eye irritation study in the rabbit:

Reference: *Smith, K. D.; 1990;* Report No. 90/ISK176/1309

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-4. The study is considered acceptable.

GLP: yes

Material and Methods:

Six male New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England, weighing 2190 - 2570 g) received 0.1 ml IKF-1216 50 % SC formulation (batch no.K15-6002, 53.15 g a.i./100 ml distilled water), diluted 1:1000 v/v in distilled water, instilled into the right eye. The left eye remained untreated (control eye). Immediately after instillation of the formulation, an assessment of the initial pain reaction of the rabbits was made. The eyes were examined and assessed at least twice during the first hour and at regular intervals throughout the day to ensure no severe injury passed un-noticed. Ocular reactions to treatment were assessed one hour, 24, 48 and 72 hours after instillation. The animals were checked daily to ensure the treated eyes were not subject to infection or causing distress.

Findings:

Instillation of the test material caused no initial pain response. The eyes of all rabbits were overtly normal throughout the 72 hour observation period.

Conclusion:

Under the conditions of this test, IKF-1216 50 % SC formulation, diluted 1:1000 v/v in distilled water, was classified “non-irritant” to the rabbit eye.

Fluazinam 500 SC: Eye irritation study in rabbits:

Reference: *Ueda, H.; 2003;* Report No. IET 02-0111

Guideline: The study was conducted according to OECD Guidelines for Testing of Chemicals (No. 405, 1987), Japanese MAFF Test Guidelines for Toxicology Studies (NohSan No. 8147, 2000) and U.S. EPA OPPTS 870.2400 (1998). The study is considered acceptable.

GLP: yes

Material and Methods:

9 female New Zealand White Rabbits (source: Minowa Breeding Center), weighing 1899 – 2305 g, were used in this study. A single dose of 0.1 ml Fluazinam IKF-1216 500SC (Batch No. 0/0001014, 39.89 w/w %) was administered to the left eye of the rabbits. 3 animals were assigned to Group A (without eye irrigation after application. The left eyes of six rabbits (Group B) were irrigated for 30 seconds starting 30 seconds after application. The right eyes were untreated (control eye). The eyes were examined for irritation at one hour and 24, 48 and 72 hours after instillation.

Findings:

No signs of corneal irritation or of iridial irritation were observed in any animal. At one hour

after instillation, all animals in Groups A and B exhibited redness of score 1 (definite hyperemia of some blood vessels). This redness of conjunctivae completely disappeared by 48 hours in both groups. At one hour after application, all animals in Groups A and B exhibited chemosis of score 1 (some swelling above normal). This chemosis of conjunctivae completely disappeared by 24 hours in both groups. No other ocular reaction was observed.

Conclusion:

Under the conditions of this test, Fluazinam 500SC suspension formulation was slightly irritating to the eye mucosa of rabbits.

B.6.11.6 Skin sensitization

ICIAO192: Skin Sensitization to the guinea pig of a 500 g/l SC formulation:

Reference.: *Lees D. et al, 1991;* Report No. CTL/P/3227

The study was performed according to the method of Buehler (*Buehler, 1965*), and in compliance with GLP principles. The study is considered acceptable.

Material and method:

500 g/l SC formulation (Fluazinam 500SC) of IKF-1216 (ICIA0192) from sample reference RS 399/H and formulation reference YF7604B contained 39.6 % w/w IKF-1216 (ICIA0192) was used in this study. This study was conducted according to the Buehler method to determine the potential of the formulation to produce dermal sensitization in guinea pigs.

Thirty female Albino Dunkin-Hartley guinea pigs (source: Harlan Porcellus, Sussex) were used in the main study after determination of the appropriate challenge doses.

For the induction procedure, the undiluted formulation (0.4 ml) was applied to a lint pad which was covered with a patch of adhesive tape and held in place by an adhesive bandage wrapped once only with PVC tape. Control animals were left untreated but an occlusive dressing was used as for the treated animals. Due to the severity of the irritation, another induction for the test animals was performed with a 50% w/v preparation in deionized water. The occlusive dressing was left in place for approximately six hours. The induction procedure was repeated at the same site, during the next two weeks at seven day intervals, for a total of three six-hour exposures. The animals were left untreated for two weeks after the final induction exposure prior to challenge.

At challenge, a 30%, a 10%, a 3% and a 1% w/v preparation of the formulation in deionized water were applied to each lint pad. The dressing was placed on the guinea pig so that the 30% formulation lint pad was on the top left shorn flank, the 10% formulation lint pad was on the bottom left shorn flank, the 3% formulation lint pad was on the top right shorn flank and the 1% formulation lint pad was on the bottom right shorn flank. It was held in place by adhesive impermeable polyethylene tape. The patches were left in place for approximately six hours. After identifying the application sites, the sites were washed with methylated spirits and water.

Formaldehyde (40 % w/v aqueous solution) was tested as a positive control. The solution was applied as a 30% and 10% w/v dilution in deionized water for the induction phase and

as a 30% w/v dilution in deionized water for the challenge phase.

Findings:

Signs of moderate skin irritation (erythema, desquamation, thickening, edema, slight hair loss and scabbing) were seen during the induction phase on all test animals. No signs of irritation were observed on any of the control animals.

Following challenge of previously induced guinea pigs with a 30% w/v preparation of the formulation in deionized water, scattered mild redness was seen in six of the 20 test animals. No response was seen in any of the control animals. The net percentage response was calculated to be 30%.

Following challenge of previously induced guinea pigs with a 10% w/v preparation of the formulation in deionized water, scattered mild redness was seen in one of the 20 test animals. No response was seen in any of the control animals. The net percentage response was calculated to be 5%.

Following challenge of previously induced guinea pigs with a 3% or a 1% w/v preparation of the formulation in deionized water, no response was seen in any of the test or control animals.

With the positive control, formaldehyde, signs of moderate skin irritation (erythema, desquamation, thickening, edema and scabbing) were seen during the induction phase on all test animals. No signs of irritation were observed on any of the control animals.

Following challenge of previously induced guinea pigs with a 30% w/v preparation of the formaldehyde solution (40% w/v in deionized water), scattered mild redness to intense redness and swelling was seen in 19 of the 20 test animals. No response was seen in any of the control animals. The net percentage response was calculated to be 95%.

Conclusion:

Using the sensitization method of Buehler, guinea pigs challenged with a 30% w/v preparation of formulation in deionized water elicited a moderate skin sensitization response, challenge with a 10% w/v preparation of formulation in deionized water elicited a mild skin sensitization response and challenge with a 3% or a 1% w/v preparation of the formulation in deionized water elicited no response.

Under the conditions of the test, 500 g/l SC formulation (Fluazinam 500SC) of IKF-1216 was a moderate skin sensitizer.

IKF-1216 50 % SC: Dermal sensitization study in guinea pigs

Reference: *Smith, K. D.; 1992;* Report No. 90/ISK159/1205

Guideline: The study was conducted according to U.S. EPA Pesticide Assessment Guidelines Subdivision F, Series 81-6. The study is considered acceptable.

GLP: yes

Material and Methods:

This study was conducted according to the Magnusson and Kligman Maximization Test to determine the potential of the formulation Fluazinam 500SC (batch no. K15-6002, 53.15 g

active ingredient/100ml) to produce dermal sensitization in guinea pigs. Ten male and 10 female Albino Dunkin-Hartley guinea pigs (source: Harlan Olac Ltd., Oxfordshire, England) were subjected to intradermal injections on Day 1 and secondary induction by occluded topical application on Day 8. The same induction procedures were carried out on a contemporaneous control group, excepting that the test material was replaced by vehicle. Positive controls used five animals per sex that received 2, 4-dinitrochlorobenzene (DNCB) using the same procedures as used to assess the test material.

For the primary induction, three pairs of 0.1 ml injections were made deep into the dermis:

Injection sites	Test Group Treatment	Control Group Treatment
Anterior sites	Freunds Complete Adjuvant (FCA)	Freunds Complete Adjuvant
Middle sites	1 % v/v IKF-1216 50 % in distilled water	Distilled water
Posterior sites	1 % v/v IKF-1216 50 % in distilled water + FCA	Distilled water + FCA

For the secondary induction, the dermal areas treated on Day 1 were treated on Day 8 by topical application of 0.6 mL 10% v/v IKF-1216 50% in distilled water in test animals. Controls received 0.6 ml distilled water. Each dose was absorbed onto an absorbent patch and was covered by an occlusive dressing for 48 hours.

The animals were challenged on Day 22 by occluded topical application of 0.03 ml distilled water to the left flank and while the right flank received 0.03 ml of 1% v/v IKF-1216 50% in distilled water at one site and 0.3% v/v IKF-1216 50% in distilled water at a second site. The occlusive dressings were removed 24 hours after challenge.

Findings:

Challenge application of 1% v/v IKF-1216 50 % in distilled water caused a significant dermal reaction (slight erythema or a more marked response) in 13 test animals. No reaction was observed in control animals.

Challenge application of 0.3% v/v IKF-1216 50 % in distilled water caused a significant dermal reaction in eight test animals. No reaction was observed in control animals. Similar application of distilled water alone caused a significant reaction in two test and no control animals.

Challenge application of 0.1% w/v DNCB in acetone elicited eight significant dermal reactions in the positive control animals. This response is consistent with that expected fro this allergen.

Conclusion:

Under the conditions of this study, repeated administration of Fluazinam 500SC caused dermal sensitization in guinea pigs.

B.6.11.7 Summary of toxicity studies with the formulation

After acute oral and dermal toxicity tests with a 50 % suspension formulation of B-1216 (fluazinam technical) the results demonstrate a low acute toxicity in Sprague-Dawley rats with LD_{50} values greater than 2000 mg/kg bw both after oral and dermal administration.

The acute inhalation LC_{50} (4 hours aerosol exposure) for the 50 % suspension formulation in male and female rats was greater than the maximum achievable concentration of the

formulation, 0.55 mg/l/4 h (snout only exposure) and 1.15 mg/l/4 h (whole body exposure), respectively.

The 50 % suspension formulation provoked slight to well defined dermal irritant effects in rabbits under the experimental conditions. However, the mean values for erythema and edema recorded 24, 48 and 72 hours after treatment did not equal or exceed the EEC limit values considered to indicate a significant inflammatory response to treatment, so classification would not be warranted.

Eye irritation studies with the 50 % suspension formulation showed mild irritation to the eye of New Zealand White rabbits (*Southwood, J.; 1985;* Report No. CTL/P/1238; *Ueda, H.; 2003;* Report No. IET 02-0111). However, in an invalid Eye irritation study (*Smith, K. D.; 1990;* Report No. 90/ISK158/1281) severe irritant effects to the rabbit eye were observed, but due to the fact that only one animal was used in this study, it was not significant for a classification. So in accordance with current EU guidelines, classification would not be warranted.

The 50 % suspension formulation induced a skin sensitization reaction in guinea pigs (both methods of Buehler and Magnusson and Kligman Maximization Test). In accordance with current EU guidelines, classification is warranted.

A summary of the results from the acute toxicity studies with the 50 % suspension formulation is presented in table 6.11.7-1.

According to Annex VI of the EC Council Directive 67/548/EEC, the 50 % suspension formulation has to be classified as sensitizing (hazard symbol Xi, risk phrase R 43).

Table 6.11.7-1 Summarised results of the acute toxicity studies with the formulation

Type of study	Species	Results	Reference
Acute oral toxicity	Rat (SPF albino; ♂/♀)	> 2000 mg/kg bw (♂/♀)	*Barber; 1985*
Acute dermal toxicity	Rat (SPF albino; ♂/♀)	> 2000 mg/kg bw (♂/♀)	*Barber; 1985*
Acute dermal toxicity	Rat (Sprague Dawley; ♂/♀)	> 2000 mg/kg bw (♂/♀)	*Cummins; 1990*
Acute inhalative toxicity	Rat (Sprague Dawley; ♂/♀)	> 0.55 mg/l (4 h) (♂/♀)	*Cracknell; 1991*
Acute inhalative toxicity	Rat (Sprague Dawley; ♂/♀)	> 1.15 mg/l (4 h) (♂/♀)	*Ebino; 2001*
Dermal irritation	Rabbit (♂) (New Zealand White)	Non-irritating	*Smith; 1990*
Dermal irritation (1:500 dilution)	Rabbit (♂) (New Zealand White)	Non-irritating	*Smith; 1990*
Dermal irritation (1:1000 dilution)	Rabbit (♂) (New Zealand White)	Non-irritating	*Smith; 1990*
Dermal irritation	Rabbit (♂) (New Zealand White)	Non-Irritating	*Southwood; 1985*

Type of study	Species	Results	Reference
Eye irritation	Rabbit (♀) (New Zealand White)	Mildly irritating	*Southwood; 1985*
Eye irritation	Rabbit (♂) (New Zealand White)	Severely irritating (limited validity, one animal only)	*Smith; 1990*
Eye irritation (1:500 dilution)	Rabbit (♂) (New Zealand White)	Non-Irritating	*Smith; 1990*
Eye irritation (1:1000 dilution)	Rabbit (♂) (New Zealand White)	Non-Irritating	*Smith; 1990*
Eye irritation	Rabbit (♀) (New Zealand White)	Non-Irritating	*Ueda; 2003*
Skin sensitisation (Buehler test)	Guinea pig (♀) (Dunkin Hartley)	Sensitizing	*Lees; 1991*
Skin sensitisation (Magnusson and Kligman)	Guinea pig (♂/♀) (Dunkin Hartley)	Sensitizing	*Smith; 1992*

B.6.12 Dermal absorption (Annex IIIA 7.3)

Dermal absorption of [^{14}C]-fluazinam in two formulations in the rat

Reference: *Roper C. S., Madden S. 2001*; Report No. 19612

The study was performed in accordance with the recommendations of the draft OECD guideline 427 for *in vivo* percutaneous absorption measurement and EPA Guideline OPPTS 870.7600, Dermal Penetration and is in compliance with GLP principles. The study is scientifically valid and therefore acceptable.

Material and method:

In this *in vivo* study, the percutaneous absorption of fluazinam was investigated in 24 male rats (Sprague Dawley; source: Charles River, UK). [^{14}C]-phenylring-radiolabelled fluazinam (batch no. 96-J29, radiochemical purity 96.44 %) and fluazinam technical (batch no. O/0001036) were used in this study. The absorption process, over 48 hours, was investigated with rat skin samples for both an undiluted [^{14}C] phenylring-radiolabelled fluazinam 500 g/l suspension concentrate formulation and a 1:1000 aqueous dilution of this formulation.

The objectives of the study were to elucidate the extent of percutaneous absorption of the compound related radioactivity, its permeation through the skin into the body and its elimination via the excreta after exposure times of 6 hours. [^{14}C]-fluazinam was applied onto a dorsal area of about 10 cm^2, limited by an O-ring under a non-occlusive protective device (gauze and rat jacket). The high dose (100 µl formulated [^{14}C]-fluazinam at a concentration of 500 mg/ml) was selected to reflect exposure to the maximal concentration possible when handling the undiluted formulation, low dose (100 µl formulated [^{14}C]-fluazinam at a 1000 times dilution of the concentrate in water) reflects exposure to a concentration recommended for use in the field.

At 6 hours post dose, the dose site skin of all animals was cleaned with cotton wool swabs soaked in “Swarfega” (industrial cleaning gel), then water and finally dried with cotton wool swabs. Groups of 4 animals each were sacrificed at 6, 24 and 48 h post dose. Urine, faeces and cage wash were collected at 6, 24 and 48 h post dose. Prior to sacrifice blood samples were taken. Following sacrifice, the O-ring was removed and retained for analysis. Stratum corneum of the dose site skin was removed by tape stripping (15 tape strips) and then the dose site skin excised.

Amounts of fluazinam present in the different samples (O-ring, cage wash, skin strips, stripped skin, whole blood and plasma, urine, faeces, gastrointestinal tract, liver and carcass) were measured by liquid scintillation counting.

Findings:

Recovery of radiolabelled test material of the applied dose for the concentrate was in a range of 98 to 105 % and 87 to 120 % for the dilution. The amounts detected in urine, faeces, cage wash, liver, GI-tract, carcass, whole blood, plasma, skin strips and stripped skin were considered as absorbed. The absorption and excretion pattern and the mean percentage of the total dose found in the different compartments are presented in table 6.12-3

Table 6.12-1 Absorption and excretion after percutaneous administration of [14C]-fluazinam (mean values expressed as % of the dose applied)

	Concentrate			Dilution		
Time group	**6h**	**24h**	**48h**	**6h**	**24h**	**48h**
Urine	0	0.01	0.02	0.01	0.04	0.06
Faeces	0	0.12	0.28	0.02	2.28	3.46
Cage wash	0	0.02	0.02	0.02	0.05	0.08
Liver	0.01	0.01	0.01	0.10	0.08	0.05
GI-tract	0.13	0.17	0.13	2.14	1.41	0.52
Carcass	0.01	0.04	0.03	0.05	0.22	0.16
Whole blood	0	0	0	0	0	0
Plasma	0	0	0	0	0	0
Skin strips	2.23	1.02	0.82	2.67	1.60	2.82
Stripped skin	0.61	0.14	0.16	1.34	0.40	0.36
Skinwash	92.65	94.96	98.61	91.70	99.79	82.29
Protective device*	4.85	4.95	2.76	4.11	2.53	3.45
Total absorbed**	**2.99**	**1.53**	**1.47**	**6.35**	**6.08**	**7.51**
Total recovery	100.49	101.44	102.84	102.16	108.4	93.25

* O-ring, gauze, rat jacket; **urine, faeces, cage wash, liver, GI-tract, carcass, whole blood, plasma, skin strips, stripped skin

Within the 6 hour exposure period, 2.99 % and 6.35 % of the dose applied was absorbed in the high and low dose, respectively. After 24 hours, a total of 1.53 % and 6.08 % of the high and low dose had been absorbed, respectively. After 48 hours, the absorption rate of the concentrate was 1.47 % and for the dilution 7.51 %. The majority of the administered dose was recovered from the skin wash (82.29 to 99.79 %). Skin stripping showed that most radioactivity at the application site was in the stratum corneum. The amount of radioactivity in skin strips, taken after the 6 hour application period and after skin wash, was 2.23 % for the concentrate and 2.67 % for the dilution of the dose applied, respectively.For the concentrate, mean radioactivity in skin strips after 24 and 48 hours was decreased (1.02 % and 0.82 %, respectively. The same applies to the amounts detected in stripped skin (0.61, 0.14 and 0.16 % after 6, 24 and 48 hours respectively, indicating further resorption from this depots.

For the dilution, most radioactivity was also detected in the stripped application site after the 6 hour exposure period (1.34 % respectively). After 24 and 48 hours, the amount of radioactivity detected in the stripped application site decreased to 0.40 % and 0.36 % respectively, indicating further resorption from this depot also.

Conclusion:

In this study, the average percutaneous absorption of [^{14}C]-fluazinam through rat skin for the undiluted formulation was in a range of 1.5 % - 3.0 %. For the diluted field formulation, the average percutaneous absorption was in a range of 6.0 % - 7.5 %.

The *In vitro* percutaneous absorption of radiolabelled fluazinam in two formulations through rat skin:

Reference: *Roper C. S,* 2000; Report No. 18992

The study was performed in accordance with the recommendations of the draft OECD guideline 428 for *in vitro* percutaneous absorption measurement and in compliance with GLP principles. The study is scientifically valid and therefore acceptable.

Material and method:

[^{14}C]-phenylring-radiolabelled fluazinam (batch no. 96-J29, radiochemical purity 96.44 %) and fluazinam technical (batch no. O/0001036) were used in this study. The absorption process, over 24 hours, was investigated with rat skin samples for both an undiluted [^{14}C] phenylring-radiolabelled fluazinam 500 g/l suspension concentrate formulation and a 1:1000 aqueous dilution of this formulation.

A total of 13 rat skin samples were excised from the dorsal regions of 9 male animals (Wistar; source: Charles River, UK) after removal of hair. After collection the skin was stored in aluminium foil at -20°C until use.

The thickness of all skin preparations were measured with a digimatic micrometer (full thickness skin samples 950 – 1980 µm; dermatome skin samples 360 – 410 µm). The skin samples were placed in flow-through automated diffusion cells (at a constant temperature of 31.4 - 32°C at skin surface) and membrane integrity was assessed by determining the

permeability coefficient (K_p) of tritiated water.

[^{14}C]-fluazinam was applied topically to the skin in two test formulations, fluazinam suspension concentrate formulation and a 1:1000 aqueous dilution of this formulation. The formulations were applied to skins at 10 µl/cm^2 giving the nominal dose levels of 5000 µg/cm^2 and 5 µg/cm^2 respectively. The high dose was selected to reflect exposure to the maximal concentration possible when handling the undiluted formulation, the low dose reflects exposure to a concentration recommended for use in the field, i.e. a 1000 times dilution of the concentrate in water.

The receptor fluid used for the water permeability assessment was sodium chloride, 0.9 % v/v. This was changed to ethanol:water (1:1, v/v) for the test material permeability measurements.

Samples of the receptor fluid were collected in hourly fractions from 0 – 6 h post dose, then 2 hourly fractions from 6 – 24 h post dose. The underside of the skin was washed (receptor rinse) with receptor fluid to remove absorbed material that had not been collected into the receptor chamber. The unabsorbed test substance was removed with a 2 % soap solution and tissue swabs. The dislodgeable dose was calculated as the sum of the skin wash, tissue swabs and cell wash. The stratum corneum was removed by a minimum of 5 successive tape strips. Radiolabelled material was extracted from the tape by mixing with methanol : chloroform (2 : 1, v/v). Amounts of fluazinam present in the different experimental compartments were measured by liquid scintillation counting.

Findings:

Recovery of radiolabelled test material of the applied dose in rat skin was 96.1 % for the concentrate and for the dilution.

Penetration of fluazinam through rat skin into the receptor fluid (mean relative skin absorption) over a 24 h period was 1.12 % (concentrate) and 14.96 % (dilution) of the dose applied. The total absorption of fluazinam (radioactivity present in receptor fluid, receptor rinse, epidermis and tape strips = stratum corneum) was 6.46 % (concentrate) and 58.71 % (dilution) of the dose applied.

The recovery of fluazinam (expressed as % of dose applied) found in the different compartments of the concentrate and the dilution are given in table 6.12-2.

Table 6.12-2: Mean % of applied dose detected in different compartments after application of the concentrate and the dilution to rat skin

	Concentrate	Dilution
Skin wash	54.01	11.29
Tissue swabs	33.38	24.87
Cell wash	2.25	1.36
Receptor fluid	1.12	14.96

Receptor rinse	0.04	0.99
Tape strips (Stratum corneum)	4.10	18.54
Epidermis	1.20	24.22
Total absorbed*	6.46	58.71
total recovery	96.1	96.23

* receptor fluid, receptor rinse, tape strips, epidermis

For the concentrate, the majority of the applied dose was removed from the surface of the skin by skin washing and a large amount was also found in tissue swabs. Regarding the amounts of fluazinam recovered in the receptor fluid, receptor rinse, stratum corneum and epidermis as systematically available, the total penetration rate was 6.46 %.
For the dilution, the majority of the applied dose was found in the epidermis and in tissue swabs (24.22 and 24.87 % resp.), followed by stratum corneum (tape strips, 18.54 %). The total penetration rate (amounts of fluazinam recovered in the receptor fluid, receptor rinse, stratum corneum and epidermis) was 58.71 % after 24 hours of exposure.

Conclusion:
For rat skin the study results obtained demonstrated that there was a minor percentage of the dose absorbed in the fluazinam suspension concentrate (6.46 %) than in the 1:1000 aqueous dilution (58.7 %).

The *In vitro* percutaneous absorption of radiolabelled fluazinam in two formulations through human skin:

Reference: *Roper C. S., Gedik L. 2000*; Report No. 18924
The study was performed in accordance with the recommendations of the draft OECD guideline 428 for *in vitro* percutaneous absorption measurement and in compliance with GLP principles. The study is scientific valid and therefore acceptable.

Material and method:
[^{14}C]-phenylring-radiolabelled fluazinam (batch no. 96-J29, radiochemical purity 96.44 %) and fluazinam technical (batch no. O/0001036) were used in this study. The absorption process, over 24 hours, was investigated with human skin samples for both an undiluted [^{14}C] phenylring-radiolabelled fluazinam 500 g/l suspension concentrate formulation and a 1:1000 aqueous dilution of this formulation.
Human breast and abdominal skin samples were obtained from the Plastic Surgery Unit, St. Johns Hospital NHS Trust, Livingston, UK. Subcutaneous fat was removed and skin was stored in aluminium foil at -20°C until use. The thickness of all skin preparations were measured with a digimatic micrometer (full thickness skin samples 1530 – 2550 µm; dermatome skin samples 200 – 410 µm). The skin samples were placed in flow-through automated diffusion cells (at a constant temperature of 31.4 - 32°C at skin surface) and membrane integrity was assessed by determining the permeability coefficient (K_p) of tritiated water.

[^{14}C]-fluazinam was applied topically to the skin in two test formulations, fluazinam suspension concentrate formulation and a 1:1000 aqueous dilution of this formulation. The formulations were applied to skins at 10 µl/cm^2 giving the nominal dose levels of 5000 µg/cm^2 and 5 µg/cm^2 respectively. The high dose was selected to reflect exposure to the maximal concentration possible when handling the undiluted formulation, the low dose reflects exposure to a concentration recommended for use in the field, i.e. a 1000 times dilution of the concentrate in water.

The receptor fluid used for the water permeability assessment was sodium chloride, 0.9 % v/v. This was changed to ethanol:water (1:1, v/v) for the test material permeability measurements.

Samples of the receptor fluid were collected in hourly fractions from 0 – 6 h post dose, then 2 hourly fractions from 6 – 24 h post dose. The underside of the skin was washed (receptor rinse) with receptor fluid to remove absorbed material that had not been collected into the receptor chamber. The unabsorbed test substance was removed with a 2 % soap solution and tissue swabs. The dislodgeable dose was calculated as the sum of the skin wash, tissue swabs and cell wash. The stratum corneum was removed by a minimum of 5 successive tape strips. Radiolabelled material was extracted from the tape by mixing with methanol:chloroform (2 : 1, v/v). Amounts of fluazinam present in the different experimental compartments were measured by liquid scintillation counting.

Findings:

Recovery of radiolabelled test material of the applied dose in human skin was 100 % for the concentrate and for the dilution.

Penetration of fluazinam through human skin into the receptor fluid (mean relative skin absorption) over a 24 h period was 0.14 % (concentrate) and 1.90 % (dilution) of the dose applied. The total absorption of fluazinam (radioactivity present in receptor fluid, receptor rinse, epidermis and tape strips = stratum corneum) was 3.84 % (concentrate) and 47.1 % (dilution) of the dose applied.

The recovery of fluazinam (expressed as % of dose applied) found in the different compartments of the concentrate and the dilution are given in table 6.12-1.

Table 6.12-3 Mean % of applied dose detected in different compartments after application of the concentrate and the dilution to human skin

	Concentrate	Dilution
Skin wash	66.19	20.13
Tissue swabs	21.87	31.32
Cell wash	8.85	1.99
Receptor fluid	0.14	1.90
Receptor rinse	0.02	0.18
Tape strips (Stratum corneum)	2.22	30.60
Epidermis	1.46	14.41

Total absorbed*	3.84	47.09
total recovery	100.75	100.53

* receptor fluid, receptor rinse, tape strips, epidermis

For the concentrate, the majority of the applied dose was easily removed from the surface of the skin by skin washing. Regarding the amounts of fluazinam recovered in the receptor fluid, receptor rinse, stratum corneum and epidermis as systematically available, the total penetration rate was 3.84 %.

For the dilution, the majority of the applied dose was found in the stratum corneum (tape strips), tissue swabs and skin wash. The total penetration rate was 47.1 % (receptor fluid, receptor rinse, stratum corneum and epidermis) after 24 hours of exposure.

Conclusion:

For human skin the study results obtained demonstrated that there was a minor percentage of the dose absorbed in the fluazinam suspension concentrate (3.84 %) than in the 1:1000 aqueous dilution (47 %).

In vitro Absorption of Technical Material through Human Epidermis:

Reference: Scott, R. C., Ward, R. J. (1985); Report No. CTL/P/1285

No specific test guideline is cited. The study is in compliance with GLP principles, but no specific guidelines are cited here also. The study is of supplementary information only.

Material and method:

Fluazinam technical, control number Y03439/003/002 (purity not specified in report) was used in this study. The *in vitro* absorption of fluazinam was measured across human epidermis in order to provide information for hazard assessments.

Glass diffusion cells in which an epidermal sheet forms a horizontal membrane separating "donor" (outer) and "receptor" chambers were used to measure skin absorption rates. An epidermal surface of 1.82 cm^2 was available for absorption. All experiments were conducted at 30 ºC. Receptor solutions were stirred. The integrity of the epidermal membranes was established by measurement of their permeability to tritiated water.

Fluazinam technical was applied as a dry powder, completely covering the membrane surface. The donor chamber was left exposed to ambient air throughout the experiment. Receptor chambers were filled with a known volume of receptor medium (50 % ethanol:water) and were sampled (0.5 ml) at regular intervals for the duration of the experiments (56 hours). Each sample was replaced by the addition of 0.5 ml of fresh receptor medium immediately after sampling. An 0.25 ml aliquot of each sample was transferred to a vial and analyzed by HPLC.

Findings:

Following application of fluazinam technical to human skin only very small amounts were detected in the receptor chamber of the glass diffusion cell, even after 50 hours of continuous contact. These low amounts made it difficult to accurately quantify the pattern

and rate of percutaneous absorption. The maximum steady state of absorption measured in the experiments was 0.014 μg/cm^2/h and the mean steady state rate for the replicate skin sample was 0.010 μg/cm^2/h.

Conclusion:

Under the experimental conditions, the rate of absorption of fluazinam technical was very slow. Not until after 8 hours of continuous contact with the skin could fluazinam be consistently detected in the receptor phase, indicating that it had penetrated the epidermis and been percutaneously absorbed. Following this time, the rate of absorption increased and a steady state rate period of absorption (mean rate = 0.010 μg/cm^2/h) up to at least 30 hours was observed.

In vitro Absorption through Human Epidermis from the 50 % Suspension Concentrate (JF9550) and Spray Dilution:

Reference: Scott, R. C., Ward, R. J. (1985); Report No. CTL/P/1284

No specific test guideline is cited. The study is in compliance with GLP principles, but no specific guidelines are cited here also. The study is of supplementary information only.

Material and method:

Fluazinam technical, control number Y03439/003/002 (purity not specified in report) was used in this study. The *in vitro* absorption of fluazinam was measured across human epidermis in order to provide information for hazard assessments.

Glass diffusion cells in which an epidermal sheet forms a horizontal membrane separating "donor" (outer) and "receptor" chambers were used to measure skin absorption rates. An epidermal surface of 1.82 cm^2 was available for absorption. All experiments were conducted at 30 ºC. Receptor solutions were stirred. The integrity of the epidermal membranes was established by measurement of their permeability to tritiated water.

Fluazinam technical was applied as a 50 % suspension concentrate (50 % ethanol:water) and as a 1:100 spray dilution in water. The concentrate was applied to the skin exposed to ambient conditions and also in an occluded mode by covering with Parafilm. The donor chamber was left exposed to ambient air throughout the experiment. Receptor chambers were filled with a known volume of receptor medium (50 % ethanol:water) and were sampled (0.5 ml) at regular intervals for the duration of the experiments (56 hours). Each sample was replaced by the addition of 0.5 ml of fresh receptor medium immediately after sampling. An 0.25 ml aliquot of each sample was transferred to a vial and analyzed by HPLC.

Findings:

Following application of fluazinam technical as a 50 % suspension concentrate to human skin to ambient conditions (non-occluded application) mean rates of absorption were 0.016 μg/cm^2/h. In the occluded mode by covering with Parafilm, mean rates of absorption were 0.159 μg/cm^2/h. As a 1:100 spray dilution from the 50 % suspension concentrate, mean rates of absorption were 0.050 μg/cm^2/h (occluded mode).

Following these three applications, a "lag period" was detected, a period of increasing

absorption before the attainment of the steady state absorption phase. The “lag times” were 4.5 hours after application of the 50 % suspension concentrate (non-occluded), 14 hours in the occluded mode and 11.5 hours after application of the 1:100 spray dilution.

Conclusion:

Under the experimental conditions, a 10-fold increase in absorption rate was observed for fluazinam technical as a 50 % suspension concentrate in the occluded mode compared to the non-occluded mode (atmospheric conditions). Obviously, occlusion of the formulation resulted in a reduction in evaporation of volatile components in the vehicle. When the vehicle was not occluded, less fluazinam was available for absorption as the vehicle dries.

The absorption rate of the 1:100 spray dilution was only a factor 3 slower than that of the 50 % suspension concentrate in the occluded mode. Very similar “mean lag times” were detected also, about 14 hours from the concentrate and about 11 hours from the spray dilution. These results indicate that fluazinam technical was absorbed slowly through epidermal membranes.

Overall conclusion on dermal absorption studies with respect to operator risk assessment:

In the *in vivo* percutaneous absorption study of [^{14}C]-fluazinam in rats, the average absorption after 6, 24 and 48 hours was in a range of 1.5 to 3 % for the concentrate and 6 to 7.5 % for the field dilution.

In the *in-vitro* skin penetration study with human skin, dermal penetration rates were in a range of 47 % of total dose applied for the spray dilution. For the concentrate, penetration rates of 3.84 % have been obtained. For rat skin, dermal penetration rates of 58.7 % for the dilution and 6.46 % for the concentrate have been obtained. So for the aqueous dilution there is practically no species difference. For the concentrate, the mean factor for species difference would be 1.7.

Taking into account the mean factor for species difference of 1.7 calculated in the *in-vitro*-study, an absorption rate of 1.5 % can be proposed for the concentrate. For the field dilution, an absorption rate of 7 % will be assumed.

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

The formulation “Fluazinam 500 SC” contains the active ingredient fluazinam (40.37 % fluazinam technical) as well as a dispersing and stabilizing agent, an antifoam and bactericide agent and thickeners. The notifier provided Material Safety Data Sheets (MSDS) for all these auxiliaries; only limited information is available concerning the toxicity of these substances.

Table 6.13-1 Toxicological data relating to the co-formulants

Coformulants	CAS Number		MSDS date	Labelling/Comments with respect to toxicology
Soprophor 4D384	119432-41-6	Dispersing agent	17/07/2001	- Oral LD_{50}: > 2000 mg/kg (rat) - irritating to eyes - non mutagenic (Chromosomal aberration test, UDS-test) Xi, R 36
Urea	57-13-6	Stabilizer	03/09/1999	- Oral LD_{50}: 14300 mg/kg (rat)
Bentopharm 20	1302-78-97	Thickener	02/01/2002	-
Rhodopol	11138-66-2	Thickener	23/04/2002	- Oral LD_{50}: > 5000 mg/kg (rat) - not irritating to skin (rabbit) - not irritating to eye (rabbit) - not sensitizing
Proxel GXL	2634-33-5	Bactericide	12/01/00	- Oral LD_{50}: 1221 mg/kg (rat) - irritating to skin (rabbit) - corrosive to eye (rabbit) - sensitizing Xn, R 22, 38, 41, 43
Antimousse SE 47	-	Antifoaming agent	27/06/2003	- Oral LD_{50}: > 5000 mg/kg (rat) - Dermal LD_{50}: > 2000 mg/kg (rat) - not irritating to skin (rabbit) - not irritating to eye (rabbit) - not sensitizing
Water	7732-18-5	Carrier	-	-

The acute toxicity profile of all co-formulants in the representative formulation is sufficiently covered by studies performed with the preparation. On the basis of all data available and according to Annex VI of the EC Council Directive 67/548/EEC, the 50 % suspension formulation has to be classified as sensitizing (hazard symbol Xi, risk phrase R 43).

B.6.14 Exposure data (Annex IIIA 7.2)

B.6.14.1 Operator exposure

The representative formulation “Fluazinam 500 SC” is a suspension concentrate and contains 500 g/l (38.8 %) fluazinam. “Fluazinam 500 SC” is an agricultural fungicide for the control of foliar blight (*Phytophthora infestans*) and protection against tuber blight in potatoes using tractor mounted sprayers for application. The application rate is 0.2 kg a.i./ha. The application water volume is 200l – 500l/ha.

The operator exposure estimates were calculated using both the German model (Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products [Uniform Principles for Operator Protections]; Mitteilungen aus der Biologischen Bundesanstalt für

Land- und Forstwirschaft, Berlin-Dahlem, no. 277) and the UK-POEM (Scientific Subcommittee on Pesticides and British Agrochemicals Joint Medical Panel., Estimation of Exposure and Absorption of Pesticides by Spray Operators [UK MAFF] 1986 and the Predictive Operator Exposure Model [POEM], UK MAFF 1992).

The assumptions for these calculations were a wide-neck container size of 5 litres, a work rate of 20 ha (BBA) and 50 ha (POEM) per day for tractor mounted boom sprayers, an operator body weight of 60 kg (POEM) and 70 kg (BBA), a 100 % absorption rate for inhalative exposure and a dermal absorption rate of 1.5 % for the concentrate and 7 % for the diluted product. Calculations were also made with respect to personal protective equipment.

Summarised results of the individual calculations are given in table 6.14.1-1 (POEM model) and 6.14.1-2 (BBA-model).

The detailed calculations are presented in appendix 1 (POEM) and appendix 2 (BBA).

Table 6.14.1-1: Exposure scenarios, POEM model with tractor application

Dermal absorbed dose (mg a.i./kg bw/day)		Inhalative absorbed dose (mg a.i./kg bw/day)		Total systemic exposure (mg/kg bw/d)	% of AOEL
Mixing/loading	Spray application	Mixing/loading	Spray application		
Tractor mounted boom sprayer (0.2 kg a.i./ha; 200l/ha; 50 ha; potato crop); without PPE					
0.005	0.048	*	0.001	0.054475	1556
Tractor mounted boom sprayer (0.2 kg a.i./ha; 200l/ha; 50 ha; potato crop); with PPE (gloves during mixing/loading and application)					
0.00025	0.008	*	0.001	0.008775	250

*inhalative exposure not included during mixing/loading according to this model

Table 6.14.1-2: Exposure scenarios, BBA model with tractor application

Mixing/loading (mg a.i./person/day)		Application (mg a.i./person/day)				Total systemic exposure (mg/kg bw)	% of AOEL
Inhalative	Dermal (hands)	Inhalative	Dermal (hands)	Dermal (head)	Dermal (body)		
Tractor mounted boom sprayer (0.2 kg a.i./ha; 20 ha; potato crop); without PPE							
0.0024	0.144	0.004	1.52	0.24	6.4	0.0103	294
Tractor mounted boom sprayer (0.2 kg a.i./ha; 20 ha; potato crop); with PPE; (gloves, standard protective garment, hood and visor)							
0.0024	0.00144	0.004	0.0152	0.012	0.32	0.0005	13

Conclusion:

The proposed systemic AOEL of fluazinam is 0.0035 mg/kg bw/d (based on the NOAEL of 1

mg/kg bw/d in the 52-week subchronic toxicity study in dogs and using an absorption rate of 35 %). For the scenarios calculated (tractor mounted boom sprayer; 0.2 kg a.i./ha; 100 % absorption rate for inhalative exposure and a dermal absorption rate of 1.5 % for the concentrate and 7 % for the spray dilution), the results of the exposure estimations according to the POEM model exceed the proposed systemic AOEL of 0.0035 mg/kg bw/d even if personal protective equipment is used (gloves). Exposure estimations with the German model exceed the proposed systemic AOEL only if no protective equipment is used. If gloves, hood + visor, boots and an overall are used, the systemic exposure will be 13 % of AOEL.

B.6.14.2 Bystander exposure

It was stated by the notifier that in view of the recommended application techniques, bystanders will be exposed only briefly and to relatively low quantities of the spray. If the exposure of a bystander is compared to an operator (working for a complete day) and proportional to the duration of exposure, it was considered unlikely that exposure of bystanders outside the treated area would exceed the AOEL.

Calculations are based on study results with direct measurement of bystander exposure for boom spray application (*Lloid and Bell, 1983)*. Following a single pass of a sprayer, mean potential dermal exposure was measured as 0.1 ml of spray on a bystander positioned at 8 m distance from the edge of the treated area. Potential inhalation exposure was measured as 0.02 ml spray/m^3.

The further assumptions were a body weight of 60 kg, a spray concentration of 1 mg fluazinam/ml, an exposure duration of 5 minutes, a respiratory rate of 3.6 m^3/h and a dermal absorption rate of 7 %. Based on these parameters, the potential dermal exposure was calculated as 0.007 mg/person and the inhalative exposure was calculated as 0.006 mg/person resulting in a total systemic burden of 0.000217 mg/kg bw/day. These results indicate that short exposure of bystanders outside the treatment areas would be 6.2 % of AOEL and should therefore not give rise to concern.

B.6.14.3 Worker exposure

B.6.14.3.1 Field studies

Fluazinam 500F on Potato – Dislodgeable Foliar Residue Study – USA in 2000

Reference: *Kenyon R. G., 2001*; Report No. 012207-2

The study was performed according to Foliar Dislodgeable Residue Dissipation US EPA OPPTS Guideline 875.2100 and is in compliance with GLP principles. The study is scientific valid and therefore acceptable.

Material and method:

Fluazinam 500F was applied with a tractor-mounted boom sprayer to potatoes at a target rate of 0.51 kg a.i./ha in 4 applications at seven day intervals for a total treatment of 2.15 kg a.i./ha. Forty leaf punches of 5.0 cm^2 (one side) were taken per sample replicate prior to and

immediately after each of the first three applications, and prior to and at 1 and 8 hours, and at 1, 2, 4, 7, 10, 14, 21, 28 and 35 days after the last application. Fluazinam residues were **dislodged** from the leaf disk surface by shaking with detergent water solution and quantified.

Findings:

Mean fluazinam residues were 0.83, 1.52, 1.46 and 1.57 µg/cm^2 after the first, second, third and fourth applications, respectively. By 7 days after each of the first three applications, the mean residue levels had decreased to 0.20, 0.49 and 0.53 µg/cm^2, respectively. Rainfall after each of the first 3 applications was 30 mm, 50 mm and 163 mm, respectively. The mean surface residue in potato leaf rinsates after 8 hours with no rainfall was 0.30 µg/cm^2 after the final application. Mean residues had decreased to < 0.01 µg/cm^2 by 35 days after the 4th application with 5.18 cm of rainfall.

The half-life for fluzinam on potato leaves can be estimated from the data obtained after the last application. Calculations using a First Order Multi Compartment model suggest a foliar dislodgeable residue half-life of 0.05 day (1.2 hours) for potatoes after 4 applications. The corresponding DT_{90} estimate was 1.17 days.

Fortified potato dislodging solutions had 13% loss in freezer storage over 59 days of freezer storage. Field fortifications had mean recoveries of 82, 72 and 77% after 179, 166 and 145 days of storage/transit time, respectively. These data indicate no significant change in stability of solutions or fortifications between 59 and 179 days of storage. The field samples had storage intervals of 139 to 206 days. Based on the stability tests, the field samples would be expected to be stable during the storage intervals.

Conclusion:

Fluazinam 500F applied to the surface of potato leaves at a concentration of 0.51 kg a.i./ha declines very rapidly, with a DT_{50} of 0.05 day (1.2 hours). Any residual fluazinam 500F on the leaf surface rapidly declines as can be shown by the calculated DT_{90} of 1.17 days.

Mean fluazinam residues one hour after the first, second, third and fourth application of 0.51 kg a.i./ha were 0.83, 1.52, 1.46 and 1.57 µg/cm^2. By 7 days after each of the first three applications, the mean residue levels had decreased to 0.20, 0.49 and 0.53 µg/cm^2, respectively.

Estimated fluazinam residues one hour after an application of 1 kg a.i./ha would be 1.6 µg/cm^2. So for the estimation of worker exposure (B.6.14.3.2), dislodgeable foliar residues (DFR) of 1.6 µg/cm^2 were assumed:

B.6.14.3.2 Estimation of worker exposure

After the application of Fluazinam 500 SC to potatoes it is not necessary to enter the field for harvesting or any other purposes. The minimum pre-harvest interval are 7 days. Any residual of the plant protection product on the leaf surface rapidly declines as has been shown in the field study (calculated DT_{90} of 1.17 days). Nevertheless, the highest potential for worker exposure following re-entry will be skin contamination. Risk of inhalation exposure would be generally confined to a very brief period after application, while the product is drying which

will be rapid under outdoor conditions.

It can be assumed that the skin contamination of workers will depend largely on the dislodgeable foliar residues (DFR) and the duration of work.

The calculations on worker exposure were performed using a model proposed by a calculation developed by industry (Krebs et al; 2000: Uniform Principles for Safeguarding the Health of Workers Re-entering Crop Growing Areas After Application of Plant Protection Products; unpublished).

The re-entry/dermal exposure (D) is calculated by the formula:

$$D = DFR \times TF \times A \times (P) \times R$$

Further assumptions for the calculations were a work rate (A) of 8 hours/day, a transfer factor (TF) of 5000 cm^2/person/day for crops with less intensive manipulation like potatoes, an application rate (R) of 0.2 kg a.i./ha and a penetration rate (P) of 5 % through clothes and gloves (PPE).

The systemic burden is calculated assuming a body weight of 70 kg and a dermal absorption rate of 7 %.

The estimated exposure of workers using no PPE to fluazinam re-entering treated crops will therefore be 0.0128 mg/kg bw/d (365 % of AOEL). Considering a penetration rate of 5 % through clothes and gloves (PPE), the estimated exposure will be 0.00064 mg/kg bw/d (18 % of AOEL).

The results of these calculations indicate that worker re-entry exposure following spraying on potato leaves does not exceed the AOEL if sprayed plants are handled with proper PPE.

Table 6.14.3.2-1: Re-entry exposure

Symbol	Definition	Quantity	Units
DFR	Foliar dislodgeable residues	1,6	µg/cm^2/kg a.i.
TF	Transferfactor	5000	cm^2/person x h
A	work rate/day	8	h/day
P	penetration through clothing	5	%
R	application rate	0,2	kg a.i./ha
D= DFR x TF x A x R		12,8	mg/person
D_{PPE}= DFR x TF x A x R x P		0,64	mg/person
AF	dermal absorption	7	%
bw	body weight	70	kg
Syst.exp.unprot.= D x AF/70 kg		0,0128	mg/kg bw
Syst.exp.prot.= D_{PPE} x AF/70 kg		0,00064	mg/kg bw

Appendix 1: **Operator exposure calculations (POEM-model)**

POEM – Tractor mounted boom sprayer with hydraulic nozzles (potatoes)					
A. Product Data					
1. Product name	Fluazinam 500 SC				
2a. Active ingredient	Fluazinam				
2b. Concentration	500 mg/ml				
3. Formulation type	SC				
4a. Main solvent					
4b. Concentration of solvent	na				
5. Maximum in-use a.i.concentration	1 mg/ml				
B. Exposure during mixing/loading					
1a. Container size	5	litres			
1b. Hand contamination/operation	0.01	ml			
2. Application dose	0.4	litres product/ha	10		kg a.i./day
3. Work rate	50	ha/day			
4. Number of operations	4	/day			
5. Hand contamination	0.4	ml/day			
6. Protective clothing	none		gloves		
7. Transmission to skin	100	%	5		%
8. Dermal exposure to formulation	0.4	ml/day	0.02		ml/day
9. Concentration of a.i.	500	mg/ml	500		mg/ml
10 Dermal exposure to a.i.	200	mg/day	10		mg/day
11. Percent absorbed	1.5	%	1.5		%
12. Absorbed dose	0.0050	mg/kg bw/day	0.000250		mg/kg bw/day
C. Exposure during spray application					
1. Application technique – Vehicle with cab boom hyrdaulic nozzles					
2. Application volume	200	litre spray/ha			
3. Volume of surface contamination	10	ml/h			
4. Distribution	Hands	Hands	Trunk	Legs	
	65	65	10	25	%
5. Clothing	None	gloves	permeable		
6. Penetration	100	10	5	15	%
7. Dermal exposure	6.5	0.65	0.05	0.375	ml/h
8. Duration of exposure	6 h				
D. PPE	none		gloves		
1. Total dermal exposure	41.55	ml/day	6.45		ml/day
2. Concentration of a.i.	1.000	mg/ml	1.000		mg/ml
3. Dermal exposure to a.i.	41.55	mg/day	6.450		mg/day
4. Percent absorbed	7	%	7		%
5. Absorbed dose	0.048	mg/kg bw/day	0.008		mg/kg bw/day
E. Inhaled exposure during spray application					
1. Inhalation exposure	0.01	ml/h			
2. Duration of exposure	6	h			
3. Concentration of a.i.	1.0	mg/ml			
4. Inhalational exposure to a.i.	0.060	mg/day			
5. Percent absorbed	100	%			
6. Absorbed dose	0.001	mg/kg bw/d			
F. Predicted exposure					
1. No gloves	0.054475	mg/kg bw/day	1556	% account of AOEL	
2. Gloves only when mixing/loading	0.049725		1420		
3. Gloves during spray application	0.013525		386		
4. Gloves during spray application & mix/loading	0.008775		250		

Appendix 2: Operator exposure calculations (BBA-model)

BBA – Tractor mounted boom sprayer with hydraulic nozzles (potatoes)						
Formulation	**Fluazinam 500 SC**					
Use rate (kg a.i./ha)	**0.2 kg a.i./ha**					
Treated surface per working day (ha)	**20 ha**					
A. Model Data						
Exposure during mixing/loading per kg a.i. handled:		2.4	mg on hands			
		0.0006	mg by inhalation			
Exposure during spray operation per kg a.i. handled:		0.06	mg on head			
		0.38	mg on hands			
		1.6	mg/on body surface			
		0.001	mg/by inhalation			
B. Potential Field Operator Exposure per Working Day (mg)						
	Dermal Exposure (mg)			Inhalation (mg)		
	Head	Hands	Body			
Activity:						
Mixing/loading						
Exposure/kg a.i. handled:		2.4		0.0006		
Exposure/person and working day:		9.6		0.0024		
Spraying						
Exposure/kg a.i. handled:	0.06	0.38	1.6	0.001		
Exposure/person and working day:	0.24	1.52	6.4	0.004		
C. Dermal Exposure Without and With Protective Clothing						
Exposed Body Parts	**No PPE**	**with PPE**				
		Penetration factor				
Mixing/loading:						
Hands	9.6	gloves	*0,01*	0.096		
Spraying:						
Hands:	1.52	gloves	*0,01*	0.0152		
Head:	0.24	Hood + visor	*0.05*	0.012		
Body:	6.4	overall	*0,05*	0.32		
D.Systemic Exposure Related to Activity						
	No PPE		**with PPE**			
Mixing/loading						
Dermal exposure	9.6		0.096		mg/person	
Dermal absorption	1,5		1,5		%	
Absorbed dose	0.144		0.00144		mg/person	
Inhalation	0.0024		0.0024		mg/person	
Absorption	100		100		%	
Absorbed dose	0.0024		0.0024		mg/person	
Total absorbed mix/load/person	0.1464		0.00384		mg/person	
Spraying						
Dermal exposure	8.16		0.35		mg/person	
Dermal absorption	7		7		%	
Absorbed dose	0.57		0.02		mg/person	
Inhalation	0.004		0.004		mg/person	
Absorption	100		100		%	
Absorbed dose	0.004		0.004		mg/person	
Total absorbed spraying/person	0.58		0.03		mg/person	
E. Total Systemic Exposure						
Systemic exposure/person	0.72		0.03		mg/person	
Systemic exposure /kg b.w. (70 kg)	0.0103		0.0005		mg/kg b.w.	
% of AOEL (0.0035 mg/kg bw/d)	294.53		13.12		%	

B.6.15 References relied on

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
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IIIA 7.1.2	Cummins H. A.	1992	IKF-1216: Acute percutaneous toxicity study in the rat [redacted] Report No.: LSR 90/ISK156/0990; GLP: yes Unpublished	Y	ISK
IIIA 7.1.3	Cracknell S.	1991	IKF-1216 50 % SC: Acute inhalation toxicity study in the rat [redacted] Report No.: 91/ISK161/0270; GLP: yes Unpublished	Y	ISK
IIIA 7.1.3	Ebino K.	2001	IKF-1216 500 SC: Acute inhalation toxicity study in rats [redacted] Report No.: IET 00-0048 GLP: yes Unpublished	Y	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIIA 7.1.4	Smith K. D.	1990	IKF-1216 50 % SC: Primary dermal irritation study in the rabbit [redacted] Report No.: 90/ISK157/1280; GLP: yes Unpublished	Y	ISK
IIIA 7.1.4	Smith K. D.	1990	IKF-1216 50 % SC (1:500 dilution): Primary dermal irritation study in the rabbit [redacted] Report No.: 90/ISK173/1282; GLP: yes Unpublished	Y	ISK
IIIA 7.1.4	Smith K. D.	1990	IKF-1216 50 % SC (1:1000 dilution): Primary dermal irritation study in the rabbit [redacted] Report No.: 90/ISK175/1284; GLP: yes Unpublished	Y	ISK
IIIA 7.1.4	Southwood J.	1985	B-1216 50 % suspension formulation: Skin irritation and eye irritation studies [redacted] Report No.: CTL/P/1238 GLP: yes Unpublished	Y	ISK
IIIA 7.1.5	Southwood J.	1985	B-1216 50 % suspension formulation: Skin irritation and eye irritation studies [redacted] Report No.: CTL/P/1238 GLP: yes Unpublished	Y	ISK
IIIA 7.1.5	Smith K. D.	1990	IKF-1216 50 % SC: Primary eye irritation study in the rabbit [redacted] Report No.: 90/ISK158/1281; GLP: yes Unpublished	Y	ISK
IIIA 7.1.5	Smith K. D.	1990	IKF-1216 50 % SC (1:500 dilution): Primary eye irritation study in the rabbit [redacted] Report No.: 90/ISK174/1283; GLP: yes Unpublished	Y	ISK
IIIA 7.1.5	Smith K. D.	1990	IKF-1216 50 % SC (1:1000 dilution): Primary eye irritation study in the rabbit [redacted] Report No.: 90/ISK176/1309; GLP: yes Unpublished	Y	ISK

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IIIA 7.1.5	Ueda H.	2003	Fluazinam 500 SC: Eye irritation study in rabbits [redacted] Report No.: IET 02-0111 GLP: yes Unpublished	Y	ISK
IIIA 7.1.6	Lees D., Robinson P.	1991	ICIAO192: Skin sensitisation t the guinea pig of a 500 g/l SC formulation [redacted] Report No.: CTL/P/3227 GLP: yes Unpublished	Y	ISK
IIIA 7.1.6	Smith K. D.	1990	IKF-1216 50 % SC: Dermal sensitisation study in guinea pigs [redacted] Report No.: 90/ISK159/1205; GLP: yes Unpublished	Y	ISK
IIIA 7.2.1.1	Chester G.	1991	Fluazinam estimation of absorbed dose and risk assessment for mixer-loader-applicators in france Imperial Chemical Industries PLC ICI Agrochemicals Report No.:RIC0840; GLP: yes Unpublished	Y	ISK
IIIA 7.2.3.1	Kenyon R. G.	2001	Fluazinam 500F on potato – dislodgeable foliar residue study – USA in 2000 Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: 012207-2 GLP: yes Unpublished	Y	ISK
IIIA 7.3	Roper C. S., Gedik L.	2000	The in vitro percutaneous absorption of radiolabelled fluazinam in two formulations through human skin [redacted] Report No.: 18924; GLP: yes Unpublished	Y	ISK
IIIA 7.3	Roper C. S.	2000	The in vitro percutaneous absorption of radiolabelled fluazinam in two formulations through rat skin Inveresk Research Tranent Report No.: 18992; GLP: yes Unpublished	Y	ISK

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IIIA 7.3	Roper C. S., Madden S.	2000	Dermal absorption of ^{14}C-fluazinam in two formulations in the rat [redacted] Report No.: 19612; GLP: yes Unpublished	Y	ISK
IIIA 7.3	Scott R. C., Ward R. J.	1985	B-1216: In vitro absorption through human epidermis from the 50 % suspension concentrate (JF9550) and spray dilution [redacted] Report No.: CTL/P/1284 GLP: yes Unpublished	Y	ISK
IIIA 7.3	Scott R. C., Ward R. J.	1985	B-1216: In vitro absorption of technical material through human epidermis [redacted] Report No.: CTL/P/1285 GLP: yes Unpublished	Y	ISK