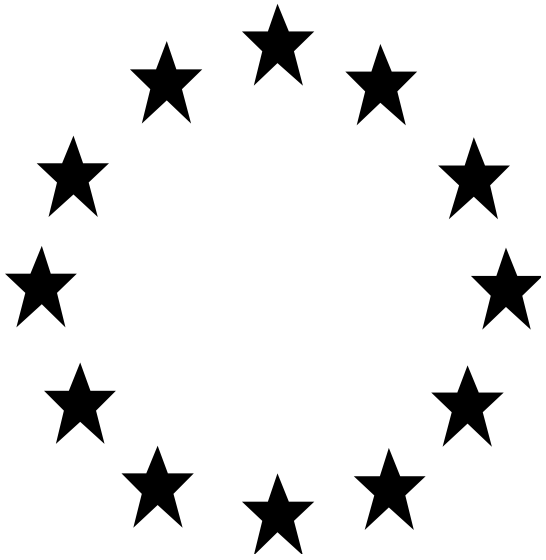


Draft Assessment Report (DAR)

- 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

Volume 3, Annex B, B.7

July 2006

Annex B

Fluazinam

B.7 Residue data

B.7 Residue data

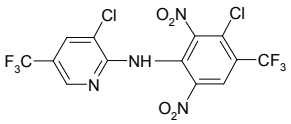
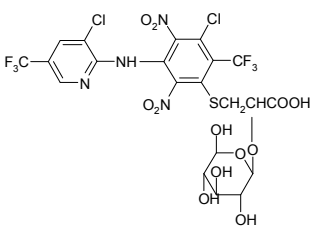
Fluazinam (IKF-1216) is a contact (foliar) fungicide developed for the preventive use in potatoes to control fungi from the class of *Oomycetes*, especially *Phytophtora infestans*. It acts by disruption in energy (ATP) production at several metabolic pathways within the fungal cell; it is also absorbed into the fungal spores, blocking various infectious processes and resulting in the death of the fungi. As a protective fungicide, fluazinam is primarily remaining on the plant surface. It is not taken up by the plant and consequently it is not translocated within the plant like systemic fungicides.

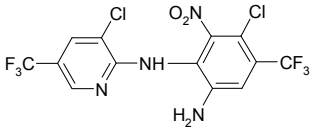
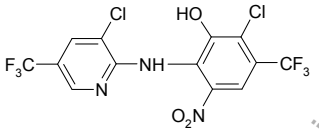
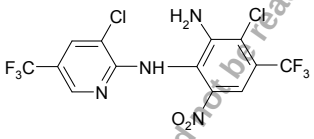
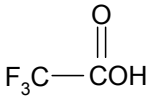
The pesticide has to be applied before the disease attack with the first application to be made when warning systems forecast significant disease attack situations. The product will be applied at a maximum individual application rate per spray of 0.2 kg active ingredient per hectare.

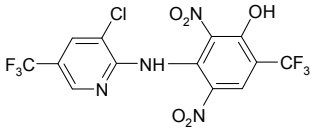
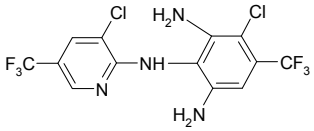
A validated analytical method for determination of fluazinam residues in various crops (potatoes, grapes, wine, onion and coffee) was submitted by the notifier; the limit of quantification (LOQ) of the method is 0.01 mg fluazinam/kg.

In table B.7-1 RMS has given an overview on parent compound, metabolites and degradation products mentioned in the metabolism sections:

Table B.7-1 Parent compound, metabolites and identified degradation products mentioned in the metabolism sections:

Chemical name (IUPAC)	Common or other name/code	Structure	Formed by	Identified in:	Maximum % TRR / ppm / (label)
3-Chloro-*N*-(3-chloro-5-trifluoromethyl-2-pyridyl)-*α,α,α*-trifluoro-2,6-dinitro-*p*-toluidine	**Fluazinam, IKF-1216**		--- (parent compound)	Potatoes, peanut (foliage), grapes, apples, laying hen (liver, kidney, muscle, fat, egg yolk), RAT	5.9 / 0.001 / (py) 7.5 / 2.3 / (py) 21.3 / 0.36 / (ph) surface wash: 42 / 1.178 / (py) liver: 2.74 / 0.027 / ph)
3-[[4-amino-3-[[3-chloro-5-(trifluoromethyl)-2-pyridyl]amino]-*α,α,α*-trifluoro-6-nitro-*o*-tolyl]thio]-2-(β-D-glucopyranosyloxy) propionic acid	**AMGT**			Potatoes grapes, wine, apples	2.7 / <0.001 / (py) 3.9 / 0.065 / (py) 12.4 / 0.051 (py) surface wash: 0.52 / 0.014 (py)

Chemical name (IUPAC)	Common or other name/code	Structure	Formed by	Identified in:	Maximum % TRR / ppm / (label)
2-(6-amino-3-chloro-*α,α,α*-trifluoro-2-nitro-*p*-toluidino)-3-chloro-5-(trifluoromethyl) pyridine	**AMPA**		Reduction (mono-amino-product of fluazinam)	Potatoes, peanut (foliage), wine goat (liver, kidney, muscle, fat, milk), laying hen (liver, kidney, muscle, fat, egg yolk and white), RAT	3.1 / <0.001 / (py) 1.6 / 0.40 / (ph) 3.7 / 0.027 / (ph) fat: 34.9 / 0.126 (py); milk: 50.9 / 0.037 / (py) fat: 81.9 / 0.767 / (ph)
2-chloro-6-[(3-chloro-5-(trifluoromethyl)-2-pyridyl)amino]-*α,α,α*-trifluoro-5-nitro-*m*-cresol	**SDS-67230**		Replacement of one nitro-group by hydroxylation	Grapes, apples	0.9 / 0.015 / (py) pomace extract: 0.36 / 0.010 / (py)
2-(2-amino-3-chloro-*α,α,α*-trifluoro-6-nitro-*p*-toluidino)-3-chloro-5-(trifluoromethyl) pyridine	**MAPA**		Reduction of one nitro-group (mono-amino-product of fluazinam, compare with AMPA; isomer)	Laying hen (liver, kidney, muscle, fat egg yolk and white)	Fat: 8.84 / 0.079 / (ph)
Trifluoroacetic acid	**TFAA**			Potatoes, peanut (foliage), apples rotational crops: lettuce (DAT 30) carrots (DAT 30) barley grain: DAT 120 DAT 365	Phenyl label only! 0.9 / <0.001 3.4 / 0.87 juice: 1.07 /0.020 95 / 0.27 82 / 0.06 74 / 0.12 59 / 0.18

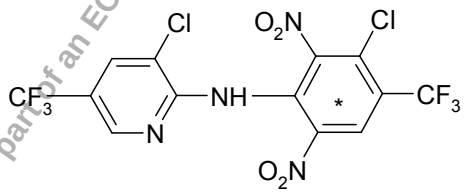
Chemical name (IUPAC)	Common or other name/code	Structure	Formed by	Identified in:	Maximum % TRR / ppm / (label)
5-[(3-chloro-5-(trifluoromethyl)-2-pyridyl)amino]-*α,α,α*-trifluoro-4,6-dinitro-*o*-cresol	**HYPA**		De-halogenation / hydroxylation of fluazinam	Laying hen (liver, kidney, muscle, fat egg yolk and white); SOIL (major)	Muscle: 5.91 / <0.01 / (py) fat: 4.3 / 0.041 / (py)
3-chloro-2-(2,6-diamino-3-chloro-*α,α,α*-trifluoromethyl-*p*-toluidino)-3-chloro-5-(trifluoromethyl) pyridine	**DAPA**		Reduction of both (two) nitro groups (diamino-product of fluazinam)	goat (liver, kidney, muscle, fat, bile, urine, milk) laying hen (liver, kidney, muscle, fat egg yolk and white), RAT	Fat: 49.2 / 0.078 / (ph) bile: 15.0 / 0.435 / (py) fat: 6.21 / 0.059 / (py)

B.7.1 Metabolism, distribution and expression of residues in plants (Annex IIA 6.1; Annex IIIA, 8.1)

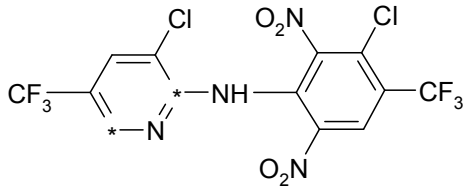
The metabolism in plants was investigated after treatment of potatoes with fluazinam representing the crop group root vegetables (1 study), on peanut plants representing the crop group pulses/oilseeds (1 study), on field grown grapes (2 studies) and on apples (1 study) representing the crop group fruits.

The plant metabolism studies were conducted using [^{14}C]IKF-1216 labelled in two positions: in the phenyl-ring and in the pyridyl-ring. The positions of the labelling are indicated by asterisks below; in the following they are designated:

[^{14}C-**B**]IKF-1216 (phenyl-ring)



[^{14}C-**Py**]IKF-1216 (pyridyl-ring)



B.7.1.1 Metabolism in potatoes

Reference: Jentoft, N. H. (1997): [^{14}C]IKF-1216 (Fluazinam) Plant Metabolism Study in Potatoes (Report No: 6775-96-0053-EF-001).

Guideline: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4.

GLP: Yes.

Study considered acceptable.

Material and Methods:

The metabolism of fluazinam (IKF-1216) in potataoes was investigated by applying ^{14}C-labelled active substance to three potato plants grown outdoors in pots in loamy sand soil. Two of the plants were treated with phenyl-labelled fluazinam ([^{14}C-**B**]IKF-1216) and one plant was treated with pyridyl-labelled fluazinam ([^{14}C-**Py**]IKF-1216). Sprayings were carried out at days 72, 86, 97 and 106 after planting.

Four foliar applications of phenyl-labelled fluazinam with an average of 0.505 kg a.i./ha (total of 2.02 kg a.i./ha) were sprayed on two plants.

Four foliar applications of pyridyl-labelled fluazinam with an average of 0.430 kg a.i./ha (total of 1.72 kg a.i./ha) were sprayed on one single plant.

The radiolabelled test material was mixed with a formulation blank to simulate treatment with a flowable formulation, which is comparable to fluazinam suspension concentrate.

The study regime is summarized in table 7.1.1-1:

Table 7.1.1-1: [14C]IKF-1216 (fluazinam) plant metabolism study in potatoes, application regime:

Plant designated as:	**Phenyl 1**	**Phenyl 2**	**Pyridyl**
Number of applications	4	4	4
Application rate (per application) [kg a.i./ha]	0.505	0.505	0.430
Application rate (total) [kg a.i./ha]	2.02	2.02	1.72
Harvest	6 days after last application	7 days after last application	7 days after last application

After harvest, tubers were air-dried and brushed lightly to remove soil. Randomly selected potatoes from each plant were pooled and homogenized for analysis. Besides, one potato from each plant was peeled with care to avoid contamination of peel and pulp, respectively, for additional studies on the distribution of residues. Further separate processing of peel and pulp followed the same order as for whole potato homogenates.

The potato homogenates were each extracted twice with 3 volumes of acetonitrile (CH_3CN); un-dissolved solids were removed by centrifugation after each extraction. The extracts of each plant were combined and concentrated for further analysis. The remaining solids were then re-extracted with three volumes of acetonitrile/water (1:1) using the same procedure and extracts from each plant combined. The final result of all extraction procedures gave two extracts and a post-extraction solid fraction (PES) per plant.

Incorporation of ^{14}C into natural products (starch) should be demonstrated by installation of starch

from the PES-fraction to sorbitol hexacetate. For this purpose, starch was first converted to glucose by hydrolysing the potato-PES in 100 mL 1N H_2SO_4 for six hours at reflux temperatures. After neutralisation, the glucose in solution was reduced to sorbitol, which then was acetylated with acetic anhydride. The reaction was stopped by adding water. After incubation overnight, sorbitol hexacetate crystals were filtered from the solution and then recrystallized to show a constant specific activity.

Findings:

The overall levels of 14C-residue were extremely low; the highest residue concentration of 0.025 mg equ/kg was found in the pyridyl-labelled fluazinam treated potatoes.The two phenyl-label treated samples had less than half of that amount of residue.

The overall distribution of radioactivity is summarized in table 7.1.1-2:

Table 7.1.1-2: Distribution of 14C-radiolabelled fluazinam in whole potato tubers

Fraction	Phenyl 1		Phenyl 2		*Phenyl average*		Pyridyl	
	[mg equ/kg]	% TRR	[mg equ/kg]	% TRR	*[mg equ/kg]*	*% TRR*	[mg equ/kg]	% TRR
Homogenate	0.0102	100.0	0.0120	100.0	*0.0111*	*100.0*	0.0250	100.0
CH3CN extract	0.0017	16.9	0.0024	19.9	*0.0020*	*18.4*	0.0079	31.5
CH3CN:H_2O (1:1)extract	0.0014	13.9	0.0025	20.7	*0.0019*	*17.3*	0.0038	15.2
Total extractable residue	0.0031	30.8	0.0049	40.6	*0.0040*	*35.7*	0.0117	46.7
PES	0.0048	47.5	0.0065	54.7	*0.0057*	*51.1*	0.0119	47.8
Total recovery [%], based on % of homogenate	78.3		95.3		*86.8*		94.5	

Since none of the extracts of the whole potato homogenates contained more than 0.0079 parent mg equ/kg, only limited identification of metabolites was possible. All extracts were subjected to reverse phase HPLC analysis to determine whether fluazinam or known metabolites of fluazinam (found in metabolism studies on peanuts, grapes and apples) such as AMPA, AMGT or TFAA represent a significant fraction of the extractable residue. The HPLC data was simplified by assigning all radioactivity that eluted rapidly to the “total polar fraction” and all the later eluting radioactivity to the “total non polar fraction”.

Comprising it can be said that radioactivity residue in potatoes consists of three fractions (see also table 7.1.1-3):

- the PES (post extraction solid), containing about half of the radioactive residue, consisting largely of potato starch. For both labellings the amount of radioactivity recovered in twice-recrystallized sorbitol hexacetate was sufficient to account for almost the entire residue in the PES fraction. It can be assumed that in potatoes (as in other crops) fluazinam is broken down

to small fragments that enter the carbon pool of the plant and are re-incorporated into natural products, mainly glucose.

- the total extractable polar residue, containing 27-31% of TRR. Since amounts were low, identification of the individual components was done for metabolites identified in the peanut metabolism study, where much higher levels were present, nevertheless allowing only the identification of the polar metabolite trifluoroacetic acid (TFAA). In the actual study TFAA, if present, represented less than 1% of the TRR, so levels were too low to confirm the presence of TFAA in potatoes. Although the remaining residue could not be identified, it is suggested that it consists of small polar fragments of fluazinam.
- the total extractable non polar residue, containing 9-16% of TRR. For the low residue levels (less than 1 µg equ/kg in the phenyl label and 4 µg equ/kg in the pyridyl label) attempts at identifying components of the fraction were limited to comparison with retention times to those of the metabolites found in the peanut metabolism study, such as AMPA and AMGT. Radioactivity assigned to fluazinam allowed estimates of parent compound of 0.3 µg equ/kg (2.3% TRR) for the phenyl labelled potatoes and 1.5 µg equ/kg (5.9% TRR) for the the pyridyl labelled potatoes. Very small amounts of radioactivity were eluted at retention times corresponding to AMGT and AMPA. Additional confirmation of assignment was done by TLC.

Table 7.1.1-3: Assignment of whole potato radioactivity to fluazinam and degradation products

Fraction	Phenyl		Pyridyl	
	[mg equ/kg] *	% TRR	[mg equ/kg] *	% TRR
Homogenate	0.011	**100**	0.025	**100**
PES **	0.006	51.1	0.012	47.8
Starch	0.005	43.9	0.012	47.3
Extracts	0.004	35.7	0.012	46.7
Total polar	0.003	27.2	0.008	30.9
TFAA	<0.001	0.9	<0.001	0.6
Total non polar	0.001	8.5	0.004	15.8
AMGT	<0.001	2.2	<0.001	2.7
AMPA	<0.001	1.4	<0.001	3.1
fluazinam	<0.001	2.3	0.001	5.9
other	<0.001	2.4	0.001	4.1

* LOQ = 0.001 mg equ/kg

** PES: post extraction solid

Additional analysis of <u>separately processed potato peel and pulp</u> resulted in very low residues for all samples and so there was considerably high variability in the amounts from one tuber to the

other. Chromatographic analysis of residues was limited to the samples from the pyridyl labelled plant due to the limited amounts of radioactivity present in the phenyl-labelled samples. Significant differences in the distribution of residues in peel compared to pulp were found only in the acetonitrile/water fraction (see table 7.1.1-4).

Table 7.1.1-4: Comparison of residue distribution in potato peel and pulp with the residue distribution in the homogenate of pyridyl labelled whole potato (data not corrected for % recoveries)

Fraction	pulp		peel		*total*		Pyridyl	
	[mg equ/kg]	% TRR	[mg equ/kg]	% TRR	*[mg equ/kg]*	*% TRR* *	[mg equ/kg]	% TRR
Total TRR	0.0159	59.3	0.0109	40.7	*0.0268*	***100.0***	0.0250	100.0
CH3CN extract	0.0046	17.3	0.0046	17.1	*0.0092*	*34.4*	0.0079	31.5
CH3CN:H_2O (1:1)extract	0.0033	12.4	0.0006	2.4	*0.0040*	*14.8*	0.0038	15.2
Total extractable residue	0.0080	29.6	0.0052	19.5	*0.0132*	*49.1*	0.0117	46.7
PES	0.0073	27.2	0.0046	17.2	*0.0119*	*44.4*	0.0119	47.8

* Total recovery: 93.6%

HPLC analysis of peel and pulp demonstrated that peel extracts contained a higher proportion of total non polar material, contributed to parent compound (identified by its retention time) than pulp; these data suggest that the non polar components, to which fluazinam is assigned, are mostly surface residues on potato.

Conclusion:

After application of phenyl ring and pyridyl ring labelled [^{14}C]-fluazinam on potato plants overall levels of ^{14}C residue in potato tubers were extremely low. The highest residue levels were found in the pyridyl level treated potatoes (25 µg equ/kg). The two phenyl label treated samples had less than half of that amount of residue. The major fractions of the TRR were:

- the PES fraction, which mostly consisted of radioactivity that had been re-incorporated into natural products such as starch,
- a polar fraction, which probably consisted of various extensively degraded fragments and soluble natural products containing re-incorporated radioactivity, and
- non polar residues in very low amounts: The total amount of non polar residue was less than 4 µg equ/kg and consisted of multiple components. The amount of parent fluazinam in all samples was less than 2 µg equ/kg.

The fact that radioactivity from both labelled fluazinam appeared in starch indicated that both rings were broken down into fragments that entered the carbon pool.

B.7.1.2 Metabolism in peanuts

Reference: Hartman, D. A. (1995): A Peanut Plant Metabolism Study with [^{14}C]IKF-1216

(Fluazinam); (Final Report), Study No: 91-0330 (Report No: 5012-91-0330-EF-002).

Guideline: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4.

GLP: Yes.

Study considered acceptable.

Material and Methods:

Peanut plants were grown from seed in loamy sand soil and later transplanted in water troughs filled with this soil. The troughs were located outdoors. In 1992, eleven plants were transplanted into three troughs (one trough was used for each label; and one for the untreated control plot). In 1993 and 1994, 27 plants were located into each of four water troughs (3 treated and 1 untreated). Because in 1992 weather conditions necessitated moving troughs into an indoor greenhouse, portable greenhouse enclosures were used later in the season for the 1993 and 1994 crops. The peanut plants were treated with four applications of 0.56 kg ^{14}C-fluazinam/ha each (total of 2.24 kg a.i./ha), to determine the nature of the terminal residue in peanut foliage, shells and nutmeat. The test material was formulated as a 40% flowable suspension to permit comparison with the use of a suspension concentrate; it was applied by mixing with water and then spraying the peanut foliage. The study regime is summarized in table 7.1.2-1:

Table 7.1.2-1: [14C]IKF-1216 (fluazinam) plant metabolism study in peanuts, application regime:

Crop of year	1992			1993				1994			
Number of troughs	3			4				4			
Treatment per trough with	[^{14}C-**B**] IKF-1216	[^{14}C-**Py**] IKF-1216	--- (control)	[^{14}C-**B**] IKF-1216	[^{14}C-**B**] IKF-1216	[^{14}C-**B**] IKF-1216	--- (control)	[^{14}C-**Py**] IKF-1216	[^{14}C-**Py**] IKF-1216	[^{14}C-**Py**] IKF-1216	--- (control)
applications	4 x 0.56 kg a.i./ha			4 x 0.56 kg a.i./ha				4 x 0.56 kg a.i./ha			
Spraying intervals in days	25, 30, 25			19, 17, 18				21, 22, 23			
Application rate total	2.24 kg a.i./ha			2.24 kg a.i./ha				2.24 kg a.i./ha			
PHI	90 days			55 days				66 days			
Sample collection	Three intermediate harvests were conducted in order to develop methodology that could be used for the final samples.			Samples were harvested when the samples were as mature as possible. Not all of the peanuts were mature. Peanuts were harvested by digging them out of the ground with a shovel. The soil was removed as far as possible and the entire plant put into plastic bags.							

The crop was split for analysis into the three RACs (foliage, shells and nutmeats).

The peanuts were shelled and the nutmeats separated from the shells.

The foliage was rinsed with water and methanol before homogenization and assayed by HPLC.

The water/methanol rinse was also analyzed.

Peanut shells were rinsed with water to remove soil remaining on the shells. The water rinse was not analyzed.

Homogenized tissue was combusted in order to determine the total radioactive residue in the three RACs. The individual RACs were analyzed using different procedures based on total residue and RAC properties.

The peanut foliage was extracted 3 times with 8:2 CH_3CN/H_2O. After each extraction the sample was centrifuged to pelletize the solids and the supernatant removed. Comparison of final extraction radioactivity with that of the first extraction indicated that extraction was complete. The extracts for the peanut foliage were combined and concentrated. The concentrated extracts were partitioned with CH_2Cl_2. Because the CH_3CN was not removed completely before partitioning, some of the organo-soluble metabolites were also found in the aqueous fraction.

The analytical methods used for foliage were not transferable to the analysis of nutmeats and shells because of the lower levels of radioactivity in them, the presence of complex matrix and the multi-component nature of the residue. For these reasons, analysis of the nutmeats concentrated on the identification and characterization of radioactivity in natural products in both the PES and organic fractions.

The aqueous CH_3CN extract was further analysed.

The peanut nutmeats were extracted 3 times with hexane, with CH_3CN and then with H_2O. Once again the supernatant from each extract was decanted after centrifugation to separate it from the PES. The hexane extracts were combined and the solvent removed at reduced pressure. The CH_3CN extract was treated similarly.

The shell extracts also contained multi-component residue that required characterization rather than direct analysis of radio-labelled natural products. The complex nature of the peanut shell natural products necessitated choosing the following route of analysis:

The peanut shells were extracted 3 times with 80/20 CH_3CN/H_2O. The supernatant was removed after centrifugation following each extraction. In some cases, the shells were further extracted with water.

Metabolites were identified using several techniques, including HPLC co-elution with standards, direct identification by mass spectrometry (MS) and comparison with standards, NMR of metabolites as well as direct comparison with standards and degradation experiments.

Findings:

As for the 1992 crop planting, growing, sampling and harvest were conducted in order to develop methodology that could be used on the final samples, only the data of 1993 and 1994 are relevant for the phenyl labelled and the pyridyl labelled crop, respectively. The results are summarized in the following tables B.7.1.2-2 and B.7.1.2-3.

Table 7.1.2-2: [14C]IKF-1216 (fluazinam) total radioactive residues in peanut plant tissues, phenyl label:

	Foliage	Nutmeats	Shells

	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]
Fluazinam	7.4	1.9	Not observed	Not observed	Not observed	Not observed
AMPA	1.6	0.40	Not observed	Not observed	Not observed	Not observed
Fatty Acids	Not applicable	Not applicable	31.5	0.23	Not applicable	Not applicable
Sucrose	Not applicable	Not applicable	9.6	0.07	Not applicable	Not applicable
Carbohydrates	12.8	3.3	5.8	0.04	3.5	0.03
Proteins	7.8	2.0	13.7	0.10	2.5	0.02
Pectin	8.9	2.3	-	<0.01	2.1	0.02
Lignin	11.2	2.9	-	<0.01	7.5	0.06
Hemicellulose	5.7	1.5	-	<0.01	9.1	0.07
Cellulose	6.3	1.6	8.2	0.06	15.6	0.12
TFAA	3.4	0.87	Not observed	Not observed	Not observed	Not observed
TFAA Derivatives	Not quantitated	Not quantitated	38.4	0.28	Not applicable	Not applicable
Glycerol	Not applicable	Not applicable	2.7	0.02	Not applicable	Not applicable
%Characterized	65.1	16.77	109.9	0.80	40.3	0.32
Other Organoextractable	5.9	1.5	1.4	0.01	7.8	0.06
Other Aqueous Extractable	18.4	4.73	1.4	0.01	46.8	0.36
Unextractable	4.3	1.1	1.4	0.01	9.1	0.07
Total	93.7	23.04	114.1	0.83	104.0	0.81

Table 7.1.2-3: [14C]IKF-1216 (fluazinam) total radioactive residues in peanut plant tissues, pyridyl label:

	Foliage		Nutmeats		Shells	
	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]
Fluazinam	7.5	2.3	Not observed	Not observed	9.3	0.40
AMPA	0.8	0.24	Not observed	Not observed	Not observed	Not observed

	Foliage		Nutmeats		Shells	
	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]	%TRR	[mg equ/kg]
Fatty Acids	Not applicable	Not applicable	48.7	0.58	Not applicable	Not applicable
Sucrose	Not applicable	Not applicable	4.2	0.05	Not applicable	Not applicable
Carbohydrates	10.4	3.2	7.9	0.09	1.8	0.08
Proteins	8.1	2.5	5.9	0.07	1.2	0.05
Pectin	6.8	2.1	1.0	0.01	2.2	0.09
Lignin	11.9	3.7	0.9	0.01	12.2	0.52
Hemicellulose	6.5	2.0	1.4	0.02	9.3	0.40
Cellulose	4.9	1.5	10.3	0.12	25.0	1.08
Glycerol	Not applicable	Not applicable	2.5	0.03	Not applicable	Not applicable
%Characterized	56.9	17.5	82.8	0.98	61.0	2.62
Other organo-extractable	18.2	5.6	6.7	0.08	11.6	0.50
Other aqueous extractable	20.2	6.2	13.4	0.16	23.0	0.99
Unextractable	2.6	0.8	-	<0.01	6.0	0.36
Total	98.1	30.1	102.9	1.22	101.6	4.47

The TRR in peanut foliage from the different labels ranged up to 30.1 mg equ/kg. The TRR in the peanut shells and nutmeats were much lower than that found in the foliage. The levels in the shells ranged up to 4.47 mg equ/kg, and the peanut nutmeats were found to have a TRR of up to 1.22 mg equ/kg.

In the **nutmeats**, fluazinam and metabolites which retained structural elements of parent compound were not found at detection limits of 0.01 mg equ fluazinam/kg. It was demonstrated that compounds of that type at levels >0.01 mg equ /kg could not be released by treatment with acids, bases or enzymes. Incorporation of ^{14}C into natural products in nutmeats was shown by a multi-step process culminating with the comparison of the isolated radioactivity with standards. The peanut nutmeats were shown for both labels to have radioactivity re-incorporated in the sucrose and the fatty acids present in this raw agricultural commodity (RAC). These levels represented approximately 5-10% (sucrose) and 30-50% (fatty acids) of the TRR.

It was also established that approximately 10% of the radioactivity in nutmeats (both labels) was present in a fraction which could not pass through a 10 000 molecular weight cutoff-membrane. This suggests that ^{14}C from fluazinam was incorporated into natural macromolecules such as

proteins. Summarized, these results establish that ^{14}C from both labels was broken down into CO_2 or other small molecules that could enter the carbon pool and be re-incorporated into natural products.

The **oil** obtained from the nutmeats was saponified either to give the free fatty acids, or for the phenyl label only, methyl esters. Before and after saponification it was also examined for the presence of fluorine. Molecules containing fluorine atoms occur very rarely in nature, therefore ^{19}F NMR was chosen as a technique that would be unique for IKF-1216 metabolites. Evidence of the complete fragmentation of the phenyl ring was seen in the observation of a trifluoroacetate signal in the ^{19}F NMR of peanut oil (phenyl label). The disappearance of this signal after saponification of the oil was accompanied by a loss of radioactivity attributed to trifluoroacetic acid (TFAA) that volatilized during workup.

The **peanut foliage** from the 1993 phenyl crop was found to contain fluazinam (1.8 mg equ/kg) and AMPA (0.4 mg equ/kg). In the 1994 pyridyl label crop, fluazinam (2.3 mg equ/kg) and AMPA were also observed (0.24 mg equ/kg). The aqueous fraction from the 1993 phenyl label foliage contained TFAA (0.87 mg equ/kg). The identity was confirmed by negative ion LC/MS and ^{19}F NMR. The extractable fractions from peanut foliage were examined under a variety of HPLC conditions. These analyses demonstrated the complex, multi-component nature of the extractable fractions.

In the case of the **peanut shells**, fluazinam was identified in the organic fraction 1992 (intermediate harvest) and 1994 pyridyl label peanut shells at 0.02 and 0.40 mg equ/kg, respectively. The variability of fluazinam in these samples was probably due to either incidental transfer of residues from the soil to the shells or direct contact to shells that were not completely underground. However, no known fluazinam metabolites were positively observed in any peanut shell samples for either label.

The metabolism of fluazinam in peanuts proceeds through AMPA, which appears to serve as an intermediate for further metabolism, since only low levels were found as residues. Some conjugation of the AMPA was evident from the results from the acid treatment of the PES and aqueous extracts. The low percentages that this metabolite represents, of the polar and PES fractions, establishes an argument for the conclusion that AMPA acts as an intermediate in the fluazinam degradative pathway.

AMPA appears to be structurally predisposed to further degradative metabolism. Since AMPA contains two adjacent phenyl ring carbons bearing NH substituents, this could represent the site of carbon bond cleavage and ring opening. Further activation of the ring toward cleavage would be achieved by further reduction or replacement of deactivating substituents by activating groups.

The appearance of radiolabeled natural products was evidence of the ultimate breakdown of fluazinam. The presence of radiolabeled TFAA also showed that indeed the molecule had broken to the extent needed for re-incorporation into natural products. The TFAA was formed by oxidative cleavage of at least two bonds in the phenyl ring. Because of this complex metabolic pathway, the metabolites formed bore very little resemblance to the parent fluazinam.

Conclusion:

The metabolism of fluazinam in peanuts showed extensive degradation and re-incorporation of the radioactivity into natural products.

In nutmeats, neither fluazinam nor any metabolites containing the phenyl-pyridyl ring structure were present in detectable amounts; radioactivity was rather incorporated into sucrose, fatty acids and proteins.

In foliage, fluazinam was detected at levels from 1.8 to 2.3 mg/kg; the reduction metabolite AMPA was also detectable at levels from 0.24 to 0.4 mg/kg. The remaining radioactivity consisted of multiple components, including trifluoroacetic acid (TFAA), indicating that extensive degradation of the fluazinam molecule had occurred.

In peanut shells only fluazinam was found at detectable levels (0.02 to 0.04 mg/kg), possibly due to the contact of shells with soil. No other residues were identified.

Fluazinam metabolism in peanuts is similar to that observed in potatoes, grapes and apples.

B.7.1.3 Metabolism in field grown grapes

References:

Flückiger, J. (1993): ^{14}C-Fluazinam: Plant Metabolism Study in Field Grown Grape; Study A (1992) [**Biological Phase**], (Report No: 328735).

Neal, T.R. (1996): ^{14}C-Fluazinam: Plant Metabolism Study in Field Grown Grape; Study A (1992) [**Analytical Phase**], Final Report, (Report No: 5431-92-0423-EF-003).

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4.

GLP: Yes.

Studies considered acceptable.

Material and Methods (**biological part**):

The purpose of the biological part of the study was the determination of the total radioactivity in grapes as raw agricultural commodity following the application of ^{14}C -fluazinam, as well as to provide grape samples for analysis and bunches of grapes for a wine fermentation study. For this purpose, field-grown grapevines of the Pinot Noir variety were treated twice with ^{14}C-labeled fluazinam at a field rate of 750 g a.i./ha. Two field plots with two vines each were separately treated with the pyridyl-labelled and the nitrophenyl-labelled test material (formulated to be representative of a 500SC fluazinam application). The first application was made at 80% of petal fall and the second at bunch closure (35 days after first application). Another plot with one plant only was laid out as a non-treated control. Harvest took place at a PHI of 71 days.

In one part of the study, bunches from the non-treated vines and the same amount of bunches from both the vines treated with the different labels were used to prepare two kinds of wine: vin de goutte and vin de presse. Fermentations were started with bunches (grapes plus stems) which had not been frozen. Bunch aliquots (about 750 g) of each sample were separately passed into a thick walled plastic bag and crushed by hand. After transfer of the samples to glass metabolism bottles equipped with sterile filters, $^{14}CO_2$ trapping systems and ventilation with nitrogen gas to avoid aerobic conditions the fermentation process was started by adding yeast-suspension to

each bottle. The fermentations were stopped when the specific gravity reached values below 1 and no more CO_2 was generated. At the end of the fermentations, each of the samples was filtered through a nylon bag (normally used for wine production) resulting in the vin de goutte samples. Each of the marcs from the filtrations was pressed to get the vin de presse. To determine the radioactivity in the nylon bags, they were extracted with methanol and, in a second step, with an acetonitrile/water solution. The methanol extracts were analysed by TLC; the bags were finally combusted for LSC. In both the vin the goutte and the vin de presse the volume, the pH and the radioactivity were determined. Preliminary TLC analyses were carried out with both kinds of wines after centrifugation; LSC analysis took place after ultrasonication and centrifugation. Samples of both kinds of wines were also submitted to a clean-up with bentonite and the resulting samples were used for the radioactivity measurements by LSC. All samples were deep-frozen at about -20 °C.

The other part of the study aimed at separating juice and pulp of about 250 g each of the bunches from the treated grapevines and the controls which were previously stored in a freezer. The bunches were chopped and crushed as described in the fermentation procedure. The juice and the pulp were separated by pressing the samples through a nylon bag. These bags were also extracted and combusted. Aliquots of each juice sample were sonicated and counted by LSC; then they were centrifuged and both the resulting supernatants and the pellets were counted. In addition, each juice sample was filtered through bentonite and measured by LSC. Preliminary TLC analyses were performed with aliquots of juices after the centrifugation and after the bentonite filtration. Each of the pulp samples was homogenised, combusted and the radioactivity was determined by LSC.

Findings (**biological part**):

The vin de goutte contained 0.535 mg parent equivalents per liter of wine (pyridyl label), 0.136 mg equ/L (nitrophenyl label) and 0.006 mg equ/L in the control samples right after the filtration step. In the vin de presse, 0.743 mg equ/L (pyridyl label), 0.645 mg equ/L and 0.006 mg parent equivalents per liter in the control sample were found after filtration. The radioactive residue in the juice was 0.331 mg equ/L. 0.540 mg equ/L were detected in the juice from the nitrophenyl label and 0.005 mg equ/L in the control juice. In the pulp, 5.152 mg parent equivalents per kg were detected for the pyridyl label, and in the case of the nitrophenyl label the corresponding value was 5.743 mg equ/kg. The radioactivity in the controls can most likely be explained by an uptake of $^{14}CO_2$ by the control plants. A summary is shown in table B.7.1.3-1. Slight decreases in the radioactivity contents were observed when the wines and the juice were ultrasonicated whereas centrifugation led to considerable reductions. Only small reductions were achieved by filtering the samples through bentonite. It has to be pointed out that the $^{14}CO_2$-free, clear wines available after the centrifugation steps best correspond to the products of common wine-makers.

The highest concentration of radioactive residue was detected in the solid deposit remaining in the nylon bags after pressing the vin de presse and the juice, respectively: the debris from the fermentations contained 6.329 mg equ/kg (pyridyl label), 5.564 mg equ/kg (nitrophenyl label) and 0.065 mg equ/kg (control). The debris from the separations into juice and pulp were found to

contain 5.152 mg equ/kg (pyridyl label), 5.743 mg equ/kg (nitrophenyl label) and 0.060 mg equ/kg (control). The $^{14}CO_2$-traps mounted after the fermentation flasks only contained 0.008 mg parent equivalents/kg from the pyridyl labelled grape bunches and 0.007 mg parent equivalents/kg from the nitrophenyl labelled grape bunches. The rate of mineralization of ^{14}C-fluazinam thus was very low.

Table 7.1.3-1: Radioactivity levels of [14C]IKF-1216 (fluazinam) in wines and juice:

Label	After filtration			After centrifugation		
	Juice [mg equ/L]	Vin de goutte [mg equ/L]	Vin de presse [mg equ/L]	Juice [mg equ/L]	Vin de goutte [mg equ/L]	Vin de presse [mg equ/L]
Pyridyl	0.331	0.535	0.743	0.208	0.455	0.442
Nitrophenyl	0.540	0.436	0.645	0.228	0.337	0.329
Control	0.005	0.006	0.006	0.004	0.005	0.005
	Pulp [mg equ/kg]					
Pyridyl	5.152					
Nitrophenyl	5.743					
Control	0.060					

Only preliminary TLC analyses of the two kinds of wines and the juice were requested by the sponsor of the study. From this analyses evidence arose that there was no parent compound in the vin de goutte nor in the vin de presse. In contrast the parent was possibly present in the juice and in the nylon bag used to separate juice and pulp of the nitrophenyl label.

To provide data on storage stability for this study, about 300 g of bunches from both labels were chopped and divided into four equal parts. The corresponding study will be reported separately.

Material and Methods (**analytical part**):

The objective of the analytical part of the study was to determine the nature and magnitude of the terminal residue occurring in grapes treated with ^{14}C–fluazinam and wine produced from these grapes.

Appropriate amounts of **grape**-berries of both the pyridyl (1 sample) and the nitrophenyl label (2 samples) harvested in the in-life phase (biological part) were placed in centrifuge bottles. Then a mixture of CH_3CN/H_2O (9:1, v /v) was added; afterwards the samples each were homogenised with a mixer for two minutes at 15000 rpm. The samples were separated by centrifugation and the liquid phases were removed. The process was repeated with 9:1 CH_3CN/H_2O and then with 1:1 CH_3CN/H_2O. The combined extracts were concentrated and subjected to liquid scintillation counting (LSC).

Radioactivity remaining in the PES was quantitated by combustion in triplicate of aliquots followed by LSC of the evolved CO_2.

The aqueous residue was extracted in sequence with hexane and with ethyl acetate. The

individual extracts were concentrated and subjected to liquid scintillation counting (LSC).
For investigation of the nature of the residue, the hexane extracts were concentrated and analysed by HPLC; radioactivity was quantitated by counting fractions collected from the HPLC run. The concentrated extract was also analysed by GC/MS in order to identify the major component. To assure that the products derived from the different labels were the same, portions from the different labels were mixed and the mixture was analysed by HPLC. The ethyl acetate (EtOAc) extracts were concentrated and analysed by HPLC; radioactivity was quantitated by counting fractions collected from the HPLC run. Fractions of the concentrated extract were purified by semi-preparative HPLC and the purified material was analysed by LC/MS. In order to assure that the products derived from the different labels were the same, portions from the different labels were mixed and the mixture was analysed by HPLC. The post-extraction solid (PES) was fractionated into six fractions consisting of various cell-wall components: water soluble polysaccharides and proteins, a starch fraction, a protein fraction, a pectin fraction, a lignin fraction, a hemicellulose fraction and a cellulose fraction. The levels of radioactivity were determined by LSC. The remaining pellets were finally measured by LSC after combustion. The aqueous phase extract was again extracted with three portions of hexane and afterwards with four portions of ethyl acetate. The aqueous phase remaining after extraction was separated into several fractions by a combination of reversed-phase, cation and anion exchange chromatography in order to record radioactivity containing sugars. All of the fractions were concentrated, and the radioactivity analysed by LSC.
Samples of grapes were also analysed to determine the level of fluazinam and AMGT by the proposed residue enforcement methods. Analysis of fluazinam was accomplished by GC with electron-capture detection; AMGT was measured by HPLC with UV detection.
The **wines** were 4 times extracted with hexane followed by four times extraction with ethyl acetate. The aqueous phase remaining after organic extraction was analysed for radioactivity by LSC. Each extract obtained was concentrated and analysed by HPLC. Radioactivity was quantitated by counting the fractions from the HPLC run. The combined hexane extract was concentrated and purified by medium-pressure liquid chromatography on a silica gel column, the resulting fractions were analysed for radioactivity by LSC. Vin de presse (nitrophenyl label) was extracted in a separate experiment with three portions hexane; the combined extract was concentrated and purified by MPLC. After further purification by HPLC the resulting products were analysed by GC/MS. Vin de presse (pyridyl label) was extracted in a third run with four portions hexane; the combined extract was concentrated and analysed by GC/MS.
The combined EtOAc extract was concentrated and purified by semi-preparative HPLC in several steps. After final purification the product was analysed by LC/MS. An aliquot of the EtOAc extract was also concentrated and heated after addition of HCl. The reaction mixture was cooled and extracted three times with EtOAc. The combined extract was concentrated and analysed by HPLC.
In order to determine whether the EtOH produced from the fermentation was labelled with 14C, samples of wine were distilled. The volume of two collected fractions and the residual liquid were

measured and the radioactivity in each sample was determined by LSC.

Findings (**analytical part**):

The TRR determined in **grape berries** was 1.16 and 1.69 mg-equ/kg from grapes treated with phenyl-label and 1.66 mg-equ/kg in grapes treated with pyridyl-label fluazinam. The extractable residues were partitioned into hexane and ethyl acetate.

The results of the analyses of residues are presented in table 7.1.3-2.

The **hexane extract** contained fluazinam as the major radioactive compound. Its concentration was found to be 0.36 and 0.19 mg-equ/kg for the nitrophenyl- and pyridyl-labelled grapes, respectively. A minor metabolite was identified as a product in which one nitro group was replaced by a hydroxyl, identified as SDS-67230 by comparison with a synthetic standard.

The **ethyl acetate extract** contained a glucose conjugate of AMPA, designated as AMGT at levels of 0 060 and 0.065 mg-equ/kg for the nitrophenyl- and pyridyl-labelled grapes, respectively.

The remainder of the radioactive residues in each of the organic extracts was accounted for as material broadly distributed over the chromatographic trace with no significant discrete radioactive peaks.

In the **aqueous phase** remaining after extraction, the most polar fraction was found to contain radioactivity that had been re-incorporated into sugars. This was indicating that extensive degradation of fluazinam followed by re-incorporation of ^{14}C into natural products had occurred.

It is reasonable to conclude that the non-discrete radioactivity in the organic, aqueous and non-extractable fractions largely represents incorporation into a variety of complex natural products as was observed in both peanuts and potatoes. This is supported by the observation that the remaining radioactivity in the aqueous phase was widely distributed over a broad range of fractions upon further chromatographic analysis.

The **PES** was subjected to a series of treatments in which the solids were degraded in stepwise fashion into six fractions representing cell wall components. Less than 5% of the radioactivity was released in each of the starch, protein and pectin fractions. The lignin, hemicellulose and cellulose fractions contained 0.1-0.2 mg-equ/kg of radioactivity. Each of these three fractions was extracted with ethyl acetate; the extracts were found to contain 0.015 up to 0.072 mg-equ/kg of radioactivity. The extracts were further analysed by HPLC; none of the resulting several HPLC-fractions amounted to more than 0.01 mg-equ/kg. There was one discrete peak found in the hemicellulose and cellulose fractions from the pyridyl-labelled sample which did not match any of the standards; however, it was present at approximately 0.01 mg-equ/kg in each of the two fractions.

Table 7.1.3-2: Distribution of radioactivity in various fractions of grapes after application of [14C]IKF-1216 (fluazinam:

Fraction	Nitrophenyl label [1)]			Pyridyl label		
	mg-equ/kg	% of fraction [2)]	% of total [3)]	mg-equ/kg	% of fraction [2)]	% of total [3)]
Initial extract	***0.96***	-	-	0.81	-	-

Fraction	Nitrophenyl label [1]			Pyridyl label		
	mg-equ/kg	% of fraction [2]	% of total [3]	mg-equ/kg	% of fraction [2]	% of total [3]
Hexane extract	0.48	100	28.4	0.32	100	19.3
IKF-1216	0.36	75.0	21.3	0.19	59.4	11.4
SDS-67230	0.0067	1.4	0.4	0.015	4.7	0.9
others [4][5]	0.12	25.0	7.1	0.13	40.6	7.8
EtOAc Extract	0.24	100	14.2	0.21	100	12.7
AMGT	0.060	25.0	3.6	0.065	31.0	3.9
others [4][5]	0.18	75.0	10.7	0.15	69.0	8.7
Aqueous phase	0.22	100	13.0	0.15	100	9.0
Sugars	0.026	11.8	1.5	0.045	30.0	2.7
others [4][5]	0.194	88.2	11.5	0.11	70.0	6.3
PES	***0.73***	-	43.2	***0.85***	-	51.2
Total	***1.69***	-	***100***	***1.66***	-	***100***

1) Data from a batch of grapes of 68.0 g

2) Based upon each fraction

3) Total: sum of initial extract and PES

4) Calculated by subtracting mg-equ/kg values of metabolites from total in extract

5) Remaining radioactivity distributed over several fractions

The TRRs determined in the samples of ***vin de presse*** produced from grapes treated with either phenyl- or pyridyl-labeled fluazinam were found to contain 0.73 mg-equ/kg of radioactive residues. *Vin de goutte* produced from grapes treated with phenyl-labeled fluazinam contained 0.41 mg-equ/kg of radioactive residues, while the *vin de goutte* from the pyridyl-labeled treatment contained 0.54 mg-equ/kg of radioactive residues.

The results of the analyses of residues are presented in table 7.1.3-3.

As mentioned before, the wine was extracted with hexanes and then ethyl acetate (EtOAc).

The **hexane extract** was found to contain AMPA as the only significant radioactive residue. Fluazinam (IKF-1216) and MAPA (isomer of AMPA) were only observed in trace amounts (<0.01 mg-equ/kg). The quantity of fluazinam isolated was not sufficient for GC/MS analysis. The assignment was based upon HPLC retention time.

The **ethyl aceate extracts** contained AMGT as the only significant radioactive product; minor

amounts of AMPA were also present. In each of the organic extracts, no other discrete metabolites that amounted to more than 1% of the TRR were observed. The remainder of the radioactive residues in each of the organic extracts was accounted for as material broadly distributed over the chromatographic trace with no significant discrete radioactive peaks.
The **ethanol** produced in the fermentation of the grapes was found to contain **radioactivity**. This result was expected because the sugars present in the grapes had been shown to contain radioactivity. It is reasonable to conclude that the non-discrete radioactivity in the organic and aqueous fractions largely represents incorporation into a variety of complex natural products.

Table 7.1.3-3: Distribution of radioactivity in wine and various fractions after application of [14C]IKF-1216 (fluazinam:

Fraction	Nitrophenyl label			Pyridyl label		
	mg-equ/kg	% of fraction [2]	% of total [3]	mg-equ/kg	% of fraction [2]	% of total [3]
Vin de presse						
Wine	***0.73***	-	100	***0.73***	-	100
Hexane extract	0.046	100	6.3	0.049	100	6.7
AMPA	0.027	58.7	3.7	0.024	49.0	3.3
others [1)2)]	0.019	41.3	2.6	0.025	51.0	3.4
EtOAc Extract	0.13	100	17.8	0.21	100	28.8
AMGT	0.053	40.8	7.3	0.076	36	10.4
AMPA	0.010	7.7	1.4	0.014	6.7	1.9
others [1)2)]	0.067	51.5	9.2	0.12	57.1	16.4
Total AMPA [3]	0.037	-	-	0.038	-	-
Aqueous phase	0.33	-	45.2	0.33	-	45.2
Ethanol	Not determined	-	Not determined	0.043	-	5.9
Vin de goutte						
Wine	***0.41***	-	100	***0.54***	-	100
Hexane extract	0.017	100	4.1	0.018	100	3.3
AMPA	0.010	58.8	2.4	0.0065	36.1	1.2
others [1)2)]	0.007	41.2	1.7	0.012	63.9	2.1
EtOAc Extract	0.13	100	31.7	0.19	100	35.2

Fraction	Nitrophenyl label			Pyridyl label		
	mg-equ/kg	% of fraction [2]	% of total [3]	mg-equ/kg	% of fraction [2]	% of total [3]
AMGT	0.051	39.2	12.4	0.065	34.2	12.0
AMPA	0.0041	3.2	1.0	0.0077	4.1	1.4
others [1][2]	0.075	57.6	18.3	0.1173	61.7	21.7
Total AMPA [3]	0.014	-	-	0.014	-	-
Aqueous phase	0.23	-	56.1	0.24	-	44.4
Ethanol	0.022	-	5.4	0.032	-	5.9

[1] Calculated by subtracting mg-equ/kg values of metabolites from total in extract

[2] Remaining radioactivity distributed over several fractions

[3] Sum of amount in hexane and ethyl acetate extracts

The processes involved in producing wine from grapes lead to significant reductions in fluazinam residues and total radioactive residues. The TRR levels in wine ranging, from 0.41 to 0.73 mg-equ/kg, represented only approximately 25-45% of the TRR levels in grapes. Since fluazinam has low water solubility, it is indicated that those residues preferentially absorb to the insoluble skins and pulp that are removed during processing.

Conclusion:

The metabolism of fluazinam in grapes is proposed to consist of reduction of a nitro group to an amino group to form AMPA, and then replacement of the phenyl chlorine with a sulphur-containing side chain, followed by attachment of glucose to form AMGT. This process is analogous to the pathway in rat metabolism that gives the cysteine conjugates of AMPA. Extensive degradation also occurs (with or without the intermediacy of AMPA and AMGT) to small fragments that are re-incorporated into natural products. The metabolic pathways are similar to those for other crops such as potatoes and peanuts. A minor metabolite representing less than 5% of the TRR in grapes was identified as AMGT, a conjugate of AMPA.

B.7.1.4 Metabolism in apple trees

References:

McClanahan, R.H. (1996): ^{14}C-IKF-1216 (Fluazinam): Plant Metabolism Study in Apple Trees; (Report No: 6021-94-0050-EF-001).

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4.

GLP: Yes.

Studies considered acceptable.

Material and Methods:

The study was conducted with [^{14}C]fluazinam under outdoor growing conditions using two

different radiolabelled tracers, ^{14}C-phenyl labelled fluazinam and ^{14}C-pyridyl labelled fluazinam. The test material was applied as a 500F (flowable) formulation (similar to Fluazinam 500SC) in a total of six applications of approximately 0.93 kg ai/ha each application for a total of 5.68 kg ai/ha. Apples were harvested 32 days after the last application, washed with acetonitrile to remove surface residues then ground with dry ice and centrifuged to yield pomace and juice. The intervals between the individual applications, chronologically listed, were 9, 22, 34, 34 and 30 days.

Pomace was extracted with acetonitrile and partitioned with ethyl acetate to generate aqueous and organic fractions.

Juice was partitioned with ethyl acetate to generate the same fractions.

Surface wash, ethyl acetate and aqueous fractions were each analyzed by high performance liquid chromatography with radioactivity detection (HPLC-RAD) to detect and quantitate the remaining fluazinam and metabolites.

Chromatographic analyses of samples from both phenyl and pyridyl labels were closely compared to determine if metabolic cleavage of the phenyl and pyridyl rings occurred. The major metabolites of fluazinam were isolated and identified by one or more of the following techniques: co-elution with authentic standards, mass spectrometric identification, derivatization followed by mass spectrometry, nuclear magnetic resonance spectrometry (NMR) or degradation techniques. The nature of the unextracted radioactivity remaining in the post-extraction solids (PES) from the pomace was determined by degradation experiments.

Findings:

The **TRR** levels in apples treated with either ^{14}C phenyl- or pyridyl-labeled fluazinam ranged from 1.9 to 2.8 mg-equ/kg, depending on the radiolabel. Levels in the pomace extract and juice were very similar between the two labels, while levels in the surface wash and pomace PES were slightly different between the two labels. The residue levels and percentage of the total radioactive residue of each fraction is shown in Table 7.1.4-1.

Table 7.1.4-1: Radioactivity levels in apples from [14C]IKF-1216 (fluazinam) treated apple-trees:

Fraction	Phenyl-label		Pyridyl-label	
	[mg-equ/kg]	[% TRR]	[mg-equ/kg]	[% TRR]
Whole Apple	1.877	100	2.802	100
Surface Wash	0.684	36.4	1.282	45.8
Juice	0.158	8.4	0.207	7.4
Pomace Extract	0.209	11.1	0.309	11.0
Pomace PES	0.827	44.1	1.003	35.8

Metabolites that were identified or characterized were classified into two types:

1) those retaining the fluazinam two-ring moiety and

2) natural products resulting from degradation of either the phenyl or pyridyl ring resulting in conversion to carbon dioxide or other small molecules and subsequent re-incorporation.

The major residue identified was parent fluazinam. Metabolites retaining the fluazinam moiety isolated from apples included AMGT and SDS-67230, although at levels <3% of the TRR. Natural products included sucrose, fructose and glucose. Degradation procedures showed that radiolabel was associated with structural polymers of apple tissue such as pectin, lignin, hemi cellulose and cellulose. Additional evidence of complete degradation was the identification of trifluoroacetic acid (TFAA). The amounts of the identified and characterized residues are summarized in Table 7.1.4-2 and Table 7.1.4-3.

Table 7.1.4-2: Distribution of radioactive residues in apple and apple fractions, phenyl label:

Component	Surface wash		Pomace extract		Apple juice		Total	
	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]
fluazinam	34.50	0.648	2.11	0.040	0.06	0.001	36.66	0.688
SDS-67230	1.90	0.036	0.36	0.007	---	n.d. 1)	2.26	0.042
AMGT	---	n.d. 1)	0.19	0.004	0.52	0.010	0.71	0.013
Sugars	---	n.d. 1)	3.43	0.064	5.16	0.097	8.59	0.161
Pectin	---	n.d. 1)	1.86	0.035	---	n.d. 1)	1.86	0.035
Lignin	---	n.d. 1)	5.95	0.112	---	n.d. 1)	5.95	0.112
Hemicellulose	---	n.d. 1)	12.02	0.225	---	n.d. 1)	12.02	0.225
Cellulose	---	n.d. 1)	6.20	0.116	---	n.d. 1)	6.20	0.116
TFAA	---	n.d. 1)	0.16	0.003	1.07	0.020	1.23	0.023
Total Characterized (%)	36.40	0.684	32.28	0.606	6.81	0.128	75.47	1.415
Other Organoextractable	---	n.d. 1)	2.49	0.047	0.49	0.009	2.98	0.056
Other Aqueous Extractable	---	n.d. 1)	1.37	0.026	0.52	0.010	1.89	0.036
Unextractable	---	n.d. 1)	14.70	0.276	---	n.d. 1)	14.70	0.276
Total	**36.40**	0.684	**50.84**	0.955	**7.82**	0.147	**95.05**	1.783

1) n.d.: not determined <0.001 mg-equ/kg (LOQ)

Table 7.1.4-3: Distribution of radioactive residues in apple and apple fractions, pyridyl label:

Component	Surface wash		Pomace extract		Apple juice		Total	
	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]
fluazinam	42.04	1.178	2.73	0.076	---	n.d. 1)	44.77	1.254
SDS-67230	2.48	0.070	0.36	0.010	---	n.d. 1)	2.84	0.080
AMGT	---	n.d. 1)	0.38	0.011	0.52	0.014	0.90	0.025
Sugars	---	n.d. 1)	2.49	0.070	3.55	0.100	6.04	0.169
Pectin	---	n.d. 1)	1.92	0.054	---	n.d. 1)	1.92	0.054
Lignin	---	n.d. 1)	7.38	0.207	---	n.d. 1)	7.38	0.207
Hemicellulose	---	n.d. 1)	12.00	0.336	---	n.d. 1)	12.00	0.336
Cellulose	---	n.d. 1)	8.63	0.242	---	n.d. 1)	8.63	0.242

Component	Surface wash		Pomace extract		Apple juice		Total	
	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]	[%TRR]	[mg-equ/kg]
TFAA [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]	n.a. [2)]
Total Characterized (%)	44.52	1.248	35.89	1.006	4.07	0.114	84.48	2.367
Other Organoextractable	1.25	0.034	1.96	0.055	1.47	0.041	4.68	0.130
Other Aqueous Extractable	---	n.d. [1)]	1.92	0.054	1.38	0.038	3.30	0.092
Unextractable	---	n.d. [1)]	4.69	0.131	---	n.d. [1)]	4.69	0.131
Total	**45.77**	1.282	**44.46**	1.246	6.92	0.193	**97.15**	2.720

1) n.d.: not determined <0.001 mg-equ/kg (LOQ)

2) n.a.: not applicable: TFAA, if formed from degradation of the pyridyl ring, would not contain radiolabel and would not be detected by HPLC with radioactivity detection.

Conclusion:

The major residue in apples treated with fluazinam was the unchanged parent, ranging from 37% to 45% of the TRR. The two metabolites of fluazinam that retained the basic structural form of the parent molecule, SDS-67230 and AMGT, were present at levels below 3% of the TRR. Radiolabelled sugars formed by re-incorporation of radioactivity accounted for 6-9% of the TRR, while structural polymeric compounds such as hemi cellulose, pectin and cellulose accounted for another 26-30% of the TRR. Trifluoroacetic acid comprised about 1% of the TRR. Unidentified extractable residues consisted of a mixture of organic and aqueous soluble metabolites that were not further characterized due to the low levels present. Unextractable residues accounted for only 5-15% of the TRR and were not further characterized

B.7.1.5 Overall conclusion on the metabolic fate on crops

The metabolism of ^{14}C-fluazinam was investigated in potatoes, peanuts, grape and apples. Chromatographic analyses of samples from both phenyl and pyridyl labels were closely compared to determine if metabolic cleavage of the phenyl and pyridyl rings occurred. Metabolites that were identified or characterized were classified into two types:
phase 1 metabolites still retaining the basic fluazinam structural two-ring moiety and
phase 2 secondary products resulting from degradation of either the phenyl or pyridyl ring resulting in conversion to carbon dioxide or other small molecules and subsequent re-incorporation.

So the following metabolic pathway can be proposed for fluazinam:
The phase 1 of the metabolic pathway of fluazinam in plants involves reduction of one or both nitro groups (to form AMPA or DAPA). This level also includes AMGT (replacement of the phenyl ring chlorine by glutathione conjugation) and SDS-67230 (replacement of one nitro group by an hydroxyle group). These and other metabolites that could result from reduction and/or displacement are now more activated toward oxidative degradation because one or more of the

deactivating groups (NO_2, Cl) have been replaced by an activating group (NH_2, OH, SR). As aromatic rings with two adjacent activating groups are susceptible to ring opening, ring cleavage occurs.

The phase 2 products of ring fragmentation are TFAA, glucose, fructose and sucrose, resulting from complete degradation of fluazinam into $^{14}CO_2$and other small molecules (2-to3-carbon fragments) that can enter the carbon pool.

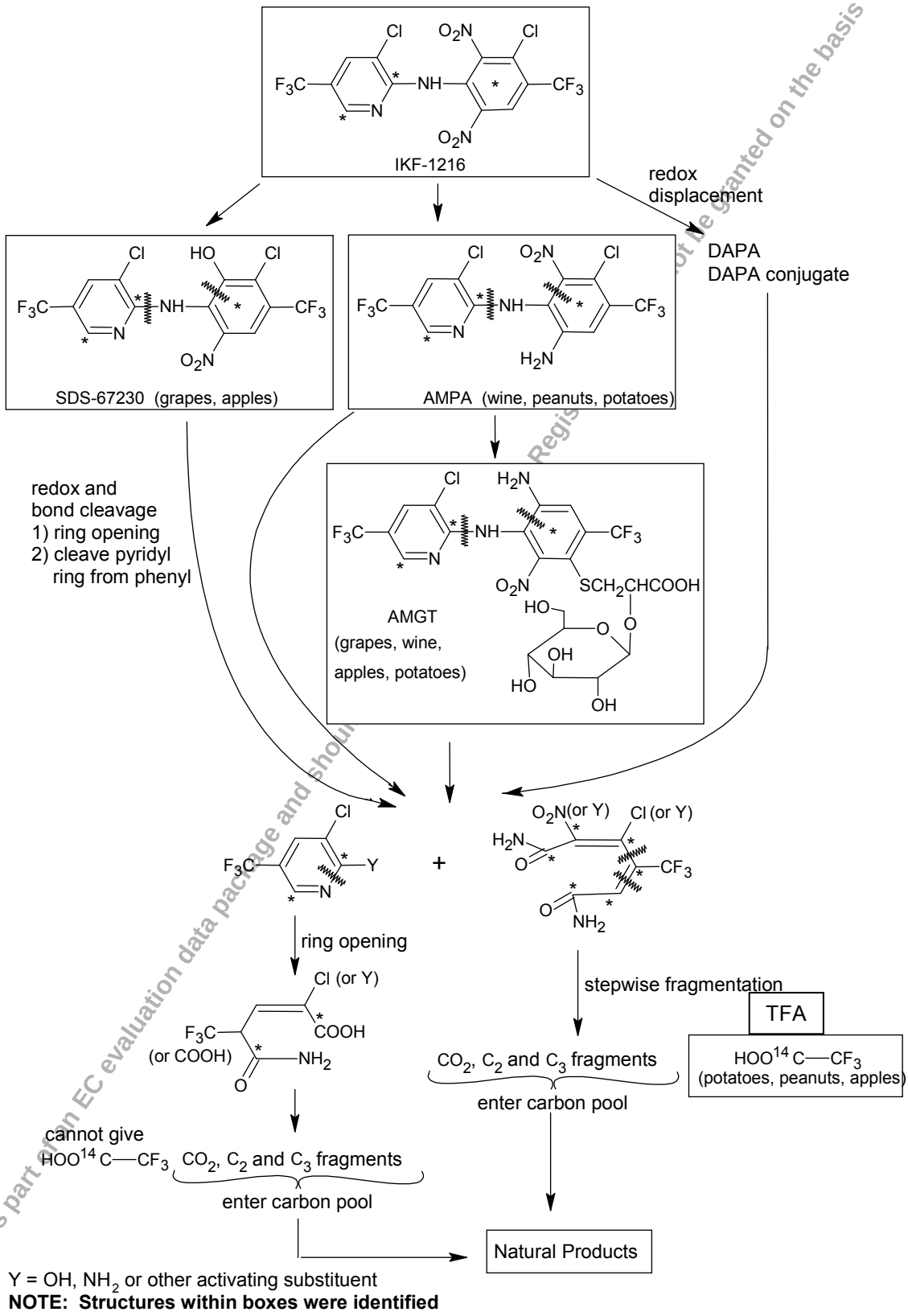
However, the secondary products of plant metabolism are numerous and complex due to the extensive metabolic processes that occur in plants. Fluazinam is the major residue on plant parts such as foliage or fruit that are exposed to the spray application. As no residues of fluazinam are observed in potato tubers or peanuts it can be concluded that fluazinam has no systemic properties.

The metabolism studies for fluazinam in potatoes, peanuts, grapes and in apples demonstrated that the end products of metabolism involved re-incorporation of ^{14}C from fluazinam into natural products, including starch and fatty acids.

In **potatoes**, the fact that radioactivity from both phenyl ring- and pyridyl ring-labelled fluazinam appeared in starch indicated that both rings were broken down into fragments that entered the carbon pool.

One product that could be identified in potatoes, peanuts, apples and rotational crops was the tri-fluoro-acetate moiety. This product can only be formed by extensive metabolism of the ring including cleavage of ring carbon-carbon bonds at two adjacent positions. The evidence indicated that initial metabolism in plants involved reduction and conjugation to give products that then were readily degraded or metabolized to release fragments which entered the carbon pool.

Fig. 7.1.5-1: Proposed metabolic pathway of fluazinam in plants:



NOTE: Structures within boxes were identified

B.7.2 Metabolism, distribution and expression of residues in livestock (Annex IIA 6.2; Annex IIIA, 8.1)

B.7.2.1 Metabolism in lactating goat

References:

Cheng, T. (1993/94): Nature of the Residue of ^{14}C- Fluazinam (IKF-1216) in Lactating Goats (Part 1: Animal Dosing, Sample Collection and Radiochemical Analysis; Part 2: Metabolite Identification and Characterization; Report No: 5248-92-0116-EF).

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4 (a)(3).

GLP: Yes.

Studies considered acceptable.

Material and Methods:

The test material, phenyl-labeled or pyridyl-labeled ^{14}C-fluazinam, was orally administered via gelatine capsule to two lactating goats once daily for four consecutive days. The phenyl-labeled test material was administered to one goat (goat 2) at 19.9 mg/day and the pyridyl-labeled test material to another goat at 19.5 mg/day (goat 3). Goat 1 received placebo capsules to serve as a control group.

Dosing: once daily, 4 days	Label	Bodyweight [kg]; mean value of days -3 and +4	Nominal dose level [mg/kg bw/day]	Average dose per day [mg]	Average feed consumption per day [kg]	Dietary concentration * [mg/kg **diet as received**]
Goat 1**	---	49	---	---	1.188	---
Goat 2	phenyl	61	0.33	19.9	1.482	13.4
Goat 3	pyridyl	54	0.36	19.5	2.130	9.15

* The data submitted for the composition of concentrate did not permit calculation of the dietary concentration on dry weight basis.

** Control animal 

Body weights were recorded on study day –10 (arrival), day –3 and day 4 (termination). Food consumption and general health and behaviour were monitored and recorded daily throughout the study. All milk, urine and faeces produced were collected and weighed twice each day. Animals were sacrificed approximately 23 hours after the last dose and select tissues (liver, kidney, fat, muscle, blood, bile, gastrointestinal tract and its contents) were collected. The total radioactive residues in each solid sample were determined by combustion and liquid scintillation counting (LSC). Liquid samples were analyzed by direct LSC.

Samples of the kidney, liver and muscle were extracted by adding acetonitrile:water (1:1) and homogenizing. After centrifuging, the resulting supernatant was separated with saturated aqueous sodium chloride and acetonitrile. The organic phase was concentrated using nitrogen and analyzed by HPLC. The aqueous phase was lyophilized to dryness and the residues

extracted (3x) with methanol containing 1% trichloroacetic acid. The combined extracts were concentrated under a nitrogen stream before HPLC analysis. Fortified control samples were also processed as described above to evaluate the extraction procedures of the treated samples.
Liver and kidney non-extractable residues and urine were enzyme-treated with either protease or β-glucuronidase/sulfatase and extracted with methanol containing 1% diethylamine. Enzyme-hydrolyzed samples were analyzed by HPLC.
Acid hydrolysis of a large-scale liver non-extractable fraction was conducted using hydrochloric acid in an attempt to isolate the hydrolysis product for identification. An elution fraction of the organic phase was extracted with hexane and purified by HPLC. Further purification of fractions with substantial radioactivity was conducted by TLC, and the major radioactivity region on the plate was scraped and extracted with acetone. The isolated acid hydrolysis product was analyzed by mass spectral (negative chemical ionization (NCI) or fast atom bombardment (FAB) mass spectroscopy) and nuclear magnetic resonance (^{1}H) analysis.
Hydrochloric acid was added to portions of bile, urine and liver organic extract and analyzed by HPLC. Aliquots of isolated metabolites and a portion of muscle aqueous phase were treated with hydrochloric acid and analyzed by HPLC.
Fat extraction consisted of homogenizing the sample in acetonitrile:water (1:1) and hexane. After centrifugation, the upper hexane phases and interphase emulsion (non-extractable solid) were separated. The lower acetonitrile:water phases were separated into organic and aqueous phases by adding saturated aqueous sodium chloride. Both phases were concentrated before chromatographic analysis. Portions of the hexane phase/non-extractable solid were suspended in ethanol/hexane/water with heating. After cooling to room temperature, the samples were filtered. Nitrogen streams were used to evaporate the upper hexane phase. The suspensions that formed were extracted three times with ethanol and the combined extracts were analyzed by HPLC.
Milk was extracted with acetonitrile (1:1 milk:acetonitrile) and the pellet extracted with acetonitrile:water (1:1). The combined extracts were separated into aqueous and organic phases with saturated aqueous sodium chloride. The organic phase was concentrated by nitrogen stream before HPLC analysis. The aqueous phase was lyophilized and extracted (3x) with methanol containing 1% trichloroacetic acid. The combined extracts were concentrated by nitrogen stream and analyzed by HPLC. Control milk was fortified and extracted as described above.
Urinary metabolites were investigated to aid in the identification or characterization of metabolites found in tissues, since the amount of material present was higher in urine and the extraction process was easier. Metabolites were isolated from urine by extracting with ethyl acetate and concentrating under vacuum. The organic phase was reduced in volume under vacuum until two phases formed. The aqueous phase was discarded. The organic phase was mixed with isopropanol and concentrated under vacuum until the organic solvent was removed. This sample was analyzed by HPLC on a reverse-phase column. Metabolites were eluted with acetonitrile and the isolated metabolites were subjected to MS analysis.
The **storage stability of milk and liver** was investigated: Milk and liver samples were stored at approximately -20°C and were extracted and analysed at about 4-month (milk) and 7-month

(liver) intervals. The metabolite profile was similar for each sample, indicating that milk and liver metabolites were stable under storage conditions for at least 4 months.

Findings:

All major components of the residues in meat, tissue and milk were identified.

There were no apparent differences in elimination and distribution of radioactivity between samples from goats administered the phenyl-labelled or pyridyl-labelled ^{14}C-fluazinam, indicating that the aryl-amine-pyridyl bond was intact. The total recovery of radioactivity in the samples collected was 86.2% in the phenyl labelled goat and 87.4% in the pyridyl labelled goat of the total radioactivity administered. Radioactivity recovered in faeces (GI contents included) accounted for 75.2% and 72.9%, respectively, in the phenyl and in the pyridyl label. The urine (cage wash included) contained 8.91% and 11.6% of the total radioactivity administered. Among edible tissues, the highest residue concentration was in the liver (0.62% and 1.21%), followed by fat (0.23% and 0.36%) in the phenyl and in the pyridyl label, respectively, with the lowest in the muscle. In other tissues, the highest radioactive residue level was in the bile followed by the GI tract and the blood.

Table 7.2.1-1: Radioactivity levels in samples from lactating goats after oral administration of [14C]IKF-1216 (fluazinam):

Sample	Phenyl label (goat 2)		Pyridyl label (goat 3)	
	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose
Liver	0.470	0.62	0.852	1.24
Kidney	0.034	<0.01**	0.060	0.01
Muscle	0.035	0.05	0.025	0.04
Fat	0.160	0.23	0.262	0.36
Total in tissues	0.699	0.90	1.199	1.65
Milk	0.018-0.071	0.31	0.018-0.078	0.59
Blood	0.015	<0.01**	0.049	<0.01**
Bile	4.660	0.08	2.901	0.16
GI tract	0.152	0.82	0.125	0.59
GI tract contents	*	9.04	*	10.51
Urine	*	8.91	*	11.55
Faeces	*	66.18	*	62.37
Total	---	86.24	---	87.42

* Not reported

** Limit of detection: 0.001 mg-equ/kg; no LOQ stated.

The entire milk production for goat 2 and 3 contained 0.31% and 0.59%, respectively. The amounts of each radiolabel found in the milk at each milking interval are shown in Table 7.2.1-2. Excluding the sample collection at sacrifice, a plateau level of fluazinam in the milk was reached quickly, representing approximately 0.08% of the dose per day in the phenyl-labelled goat and 0.15% of the dose per day in the pyridyl-labelled animal by collection days 3 and 4. The concentration of phenyl-label fluazinam in the milk ranged from 0.018 to 0.071 mg-equ/kg, and the pyridyl-label fluazinam concentration was very similar, 0.018 to 0.078 mg-equ/kg.

Table 7.2.1-2: Daily radioactivity concentrations in milk from lactating goats after oral administration of [14C]IKF-1216 (fluazinam):

Collection time	Phenyl label (goat 2)		Pyridyl label (goat 3)	
	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose
Day 1 p.m.	0.046	0.04	0.060	0.08
Day 2 a.m.	0.018	0.02	0.018	0.04
Day 2 p.m.	0.048	0.04	0.070	0.11
Day 3 a.m.	0.021	0.03	0.021	0.04
Day 3 p.m.	0.062	0.06	0.071	0.11
Day 4 a.m.	0.020	0.02	0.022	0.05
Day 4 p.m.	0.071	0.06	0.078	0.10
Sacrifice (Day 4)	0.032	0.04	0.028	0.06
Total	---	0.31	---	0.59

The radioactivity was separated into aqueous extractable, organic extractable and non-extractable solid fractions. The breakdown of each matrix into these fractions is shown in Table 7.2.1-3.

Table 7.2.1-3: Distribution of radioactivity in extractable and non-extractable fractions in samples from lactating goats after oral administration of[14C]IKF-1216 (fluazinam):

Sample	Aqueous extractable		Organic extractable		Non-extractable solid		Total %
	[mg-equ/kg]	% TRR	[mg-equ/kg]	% TRR	[mg-equ/kg]	% TRR	
Phenyl label (goat 2)							
Liver	0.048	10.3	0.082	17.5	0.302	64.2	92.0
Kidney	<0.01**	20.1	0.013	39.5	0.012	35.4	95.0
Muscle	<0.01**	4.4	0.013	37.6	0.014	39.7	81.7
Fat	<0.01**	4.2	0.127	79.9	0.012	7.5	91.6
Milk	<0.01**	3.8	0.058	83.8	<0.01	8.8	96.4
Bile	4.660	100	n.a.*	n.a.*	n.a.*	n.a.*	100
Urine	0.704	95.8	n.a.*	n.a.*	n.a.*	n.a.*	95.8
Pyridyl label (goat 3)							
Liver	0.083	9.8	0.141	16.5	0.497	58.3	84.6
Kidney	0.012	20.0	0.025	41.0	0.021	34.7	95.7
Muscle	<0.01**	5.7	0.011	43.1	0.012	47.0	95.8
Fat	<0.01**	2.6	0.195	74.3	0.012	4.5	81.4
Milk	<0.01**	3.4	0.068	93.0	<0.01**	3.0	99.4
Bile	2.901	100	n.a.*	n.a.*	n.a.*	n.a.*	100
Urine	0.909	93.3	n.a.*	n.a.*	n.a.*	n.a.*	93.3

* n.a.: not applicable

** Limit of detection: 0.001 mg-equ/kg; no LOQ stated.

Fractions amounting to more than 0.01 mg-equ/kg of radioactive residues were further characterized. In general, similar metabolite profiles were obtained for each matrix from the phenyl-labelled and the pyridyl-labelled groups. The results indicate that all detected metabolites have a structure with intact aryl-amin-pyridyl bond.

Fluazinam was not detected in any of the samples. The residues in meat, milk and tissues were mainly comprised of the reduction metabolites, AMPA and DAPA and their sulfamate conjugates. There was one sulfamate conjugate of AMPA and two sulfamate conjugates of DAPA. All of the conjugates could be converted to free AMPA and DAPA under mild hydrolysis conditions. A polar metabolite (Aq-1) present in liver, kidney, muscle and milk could be hydrolysed to DAPA using

hydrochloric acid, and was so characterized as a DAPA conjugate, although the identity of the adduct was not established. One metabolite, designated I-A, was found only in the urine and seemed to hydrolyze to DAPA upon acid hydrolysis. However, as it was not present in tissues or milk, it was not further characterized. The results of the metabolite analyses are summarized in Table 7.2.1-4.

Table 7.2.1-4: Metabolites in combined aqueous and organic extracts of samples from lactating goats after single oral administration of [14C]IKF-1216 (fluazinam) for four consecutive days

Matrix	Label	Aq-1 [1]	I-A [1]	DAPA sulfamate (I) [2]	DAPA sulfamate (II) [2]	AMPA sulfamate (III)	DAPA (IV)	AMPA (V)	Total [%]
µg ^{14}C-fluazinam equivalent per g of matrix [mg-equ/kg]									
Liver	phenyl	0.017	n.d.	0.013	0.013	0.030	0.059	n.d.	0.017
	pyridyl	n.d.	n.d.	0.013	0.026	0.047	0.074	0.064	n.d.
Kidney	phenyl	<0.01	n.d.	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
	pyridyl	0.010	n.d.	<0.01	<0.01	0.011	<0.01	<0.01	0.010
Muscle	phenyl	<0.01	n.d.	n.d.	n.d.	n.d.	<0.01	<0.01	<0.01
	pyridyl	<0.01	n.d.	n.d.	n.d.	n.d.	<0.01	<0.01	<0.01
Fat	phenyl	n.d.	n.d.	n.d.	n.d.	n.d.	0.078	0.055	n.d.
	pyridyl	n.d.	n.d.	n.d.	n.d.	n.d.	0.074	0.126	n.d.
Milk	phenyl	<0.01	n.d.	n.d.	<0.01	<0.01	0.021	0.026	<0.01
	pyridyl	<0.01	n.d.	n.d.	<0.01	0.010	0.019	0.037	<0.01
Bile	phenyl	n.d.	n.d.	3.942	n.d.	0.340	0.378	n.d.	n.d.
	pyridyl	n.d.	n.d.	2.100	n.d.	0.366	0.435	n.d.	n.d.
Urine	phenyl	n.d.	0.056	0.489	0.143	n.d.	0.016	n.d.	n.d.
	pyridyl	n.d.	0.077	0.617	0.190	n.d.	0.025	n.d.	n.d.
% of radioactivity in matrix									
Liver	phenyl	3.6	n.d.	2.7	2.7	6.3	12.5	n.d.	27.8
	pyridyl	n.d.	n.d.	1.5	3.1	5.5	8.7	7.5	26.3
Kidney	phenyl	20.1	n.d.	3.8	6.5	10.1	15.3	3.7	59.6
	pyridyl	15.9	n.d.	2.2	8.4	19.0	8.8	6.8	61.0

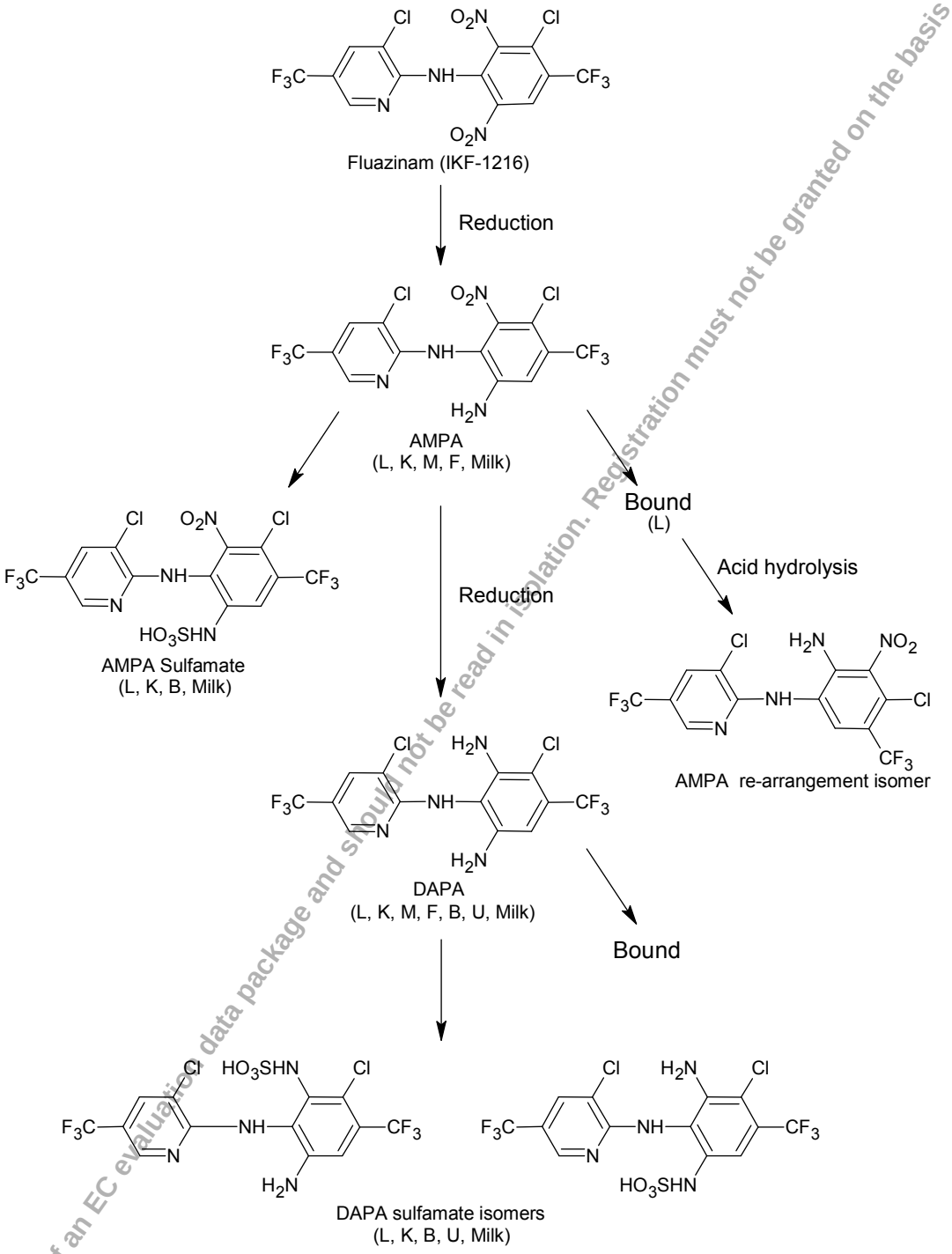
Matrix	Label	Aq-1 [1]	I-A [1]	DAPA sulfamate (I) [2]	DAPA sulfamate (II) [2]	AMPA sulfamate (III)	DAPA (IV)	AMPA (V)	Total [%]
Muscle	phenyl	4.4	n.d.	n.d.	n.d.	n.d.	17.5	20.1	42.0
	pyridyl	5.7	n.d.	n.d.	n.d.	n.d.	16.8	26.3	48.8
Fat	phenyl	n.d.	n.d.	n.d.	n.d.	n.d.	49.2	34.9	84.1
	pyridyl	n.d.	n.d.	n.d.	n.d.	n.d.	28.3	48.6	76.9
Milk	phenyl	3.8	n.d.	n.d.	4.2	11.5	30.3	37.9	87.6
	pyridyl	3.4	n.d.	n.d.	2.1	13.7	26.4	50.9	96.4
Bile	phenyl	n.d.	n.d.	84.6	n.d.	7.3	8.1	n.d.	100
	pyridyl	n.d.	n.d.	72.4	n.d.	12.6	15.0	n.d.	100
Urine	phenyl	n.d.	7.6	66.5	19.5	n.d.	2.1	n.d.	95.7
	pyridyl	n.d.	7.9	63.3	19.6	n.d.	2.6	n.d.	93.3

n.d. not detected
[1] characterised as DAPA conjugate; not further identified
[2] conjugation positions not established

Since liver and kidney contained significant amounts of non-extractable solids following initial extraction, enzymatic hydrolysis was performed using protease, sulfatase and β-glucuronidase treatments. For liver, 31 to 80% of bound radioactivity was released using the various enzyme treatments. In the case of kidney, 59 to 94% of bound radioactivity was released; however, specific metabolites could not be identified. In another experiment, the non-extractable liver fraction was subjected to strong acid hydrolysis, releasing the majority of the radioactivity into the organic extractable phase (up to 86.5% of the non-extractable radioactivity). DAPA was a minor component, while the majority of the released radioactivity was less polar metabolite which did not co-elute with any metabolite standard but was subsequently identified as a rearrangement product of AMPA. It was shown that AMPA could re-arrange to this isomer under acidic conditions.

The proposed metabolic pathway of ^{14}C-fluazinam in lactating goats is presented below.

Fig. B.7.2.1-5: Proposed metabolic pathway of fluazinam in lactating goat:



(Letters in parentheses indicate matrix in which metabolite was identified:
L=Liver, K=Kidney, M=Muscle, F=Fat, B=Bile, U=Urine)

Conclusion:

The nature of the residue studies in lactating goats show that the major residues were the reduction products AMPA and DAPA and their sulfamate conjugates. These residues were similar

to residues identified after oral administration of fluazinam to rats (the AMPA and DAPA conjugates identified from rats were glucuronides). Parent fluazinam was not detected in any of the samples. A summary of the combined residue data for goats fed fluazinam is presented in Table 6.2-6.

Table 7.2.1-6: Summary of residue data for goats fed with [14C]IKF-1216 (fluazinam) at 0.345 mg/kg bw/d diet level (average of phenyl and pyridyl label).

[mg-equ/kg]	Milk	Muscle	Fat	Liver	Kidney
TRR [1]	0.071 [2]	0.030	0.211	0.661	0.047
Extractable [3]	0.064	0.012	0.161	0.177	0.025
Non-extractable [3]	<0.01	0.013	0.012	0.400 [4]	0.017
Fluazinam	n.d.	n.d.	n.d.	n.d.	n.d.
DAPA [5, 8]	0.02	<0.01	0.076	0.099	<0.01
AMPA [6, 8]	0.04	<0.01	0.091	0.07	<0.01
Unknowns [8], (number [7])	<0.01 (0)	<0.01 (0)	n.d. (0)	0.017 (1, phenyl)	0.01 (1, pyridyl)

n.d. not detected

1 Values in table are averages for phenyl and pyridyl labels listed in table B.7.2.1-1.

2 TRR for ^{14}C milk samples used for metabolite analysis. Highest TRR levels in milk from in-life study were for day 4 pm samples with 0.075 mg-equ/kg.

3 Values in table are averages for phenyl and pyridyl labels listed in table B.7.2.1-3

4 Non-extractable residues were further characterised. The products of acid hydrolysis demonstrated that non-extractable residues mainly contained covalently bound AMPA.

5 DAPA values represent sum of DAPA and two DAPA sulfamates

6 AMPA values represent sum of AMPA and AMPA sulfamate

7 Unknowns ≥0.01 mg-equ/kg

8 Values in table are averages for phenyl and pyridyl labels listed in table B.7.2.1-4.

The plateau level of TRR in the milk was reached quickly. By calculating the following theoretical maximum transfer factors (= ratio of concentration in tissue to concentration in feed; listed in table B.7.2.1-7), RMS tries to give an idea, that in this study very low transfer into edible tissues and milk was observed. These factors, of course, could not replace transfer factors resulting of a livestock feeding study.

Table B.7.2.1-7 Theoretical maximum transfer factors for fluazinam derived TRR in ruminants, basing on the average dietary burden of phenyl and pyridyl label on fresh weight basis of 11.3 mg fluazinam/kg feed as received:

Transfer Factors	
Milk	**0.0063**
Muscle	**0.0027**
Fat	**0.0187**
Liver	**0.0585**
Kidney	**0.0042**

B.7.2.2 Metabolism in laying hen

References:

Cheng, T. (1995): Nature of the Residue of ^{14}C- Fluazinam (IKF-1216) in Laying Hens (Report No: 5309-92-0241-EF).

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4 (b)(3).

GLP: Yes.

Study considered acceptable.

Material and Methods:

Two groups of laying hens were dosed with a single gelatine capsule orally for four consecutive days at approximately 10 mg of fluazinam/kg of feed (fresh weight) using both the phenyl- and pyridyl-label test materials. Eight hens were dosed with the phenyl-labelled test material and seven with the pyridyl-labeled test material. Five control animals received capsules that contained dextrose only.

Dosing: once daily, 4 days	Number of animals	Label	Bodyweight [kg]; mean value of days -4 and +4	Nominal dose level [mg/kg bw/day]	Average dose per day [mg]	Average feed consumption per day [kg]	Dietary concentration * [mg/kg **diet as received**]
Group 1**	5	---	1.516	---	---	0.122	---
Group 2	8	phenyl	1.570	0.76	1.2	0.119	10.08
Group 3	7	pyridyl	1.583	0.76	1.2	0.113	10.62

* As no data were submitted on the composition of the basal diet, a calculation of the dietary concentration on dry weight basis was not possible.

** Control group.

All eggs and excreta were collected daily, weighed and analyzed. The animals were sacrificed approximately 6 hours after administration of the last dose and selected samples and blood were collected. Samples collected included kidney, liver, fat, skin, thigh and breast muscle, gastrointestinal tract and contents, and any shelled eggs in the oviduct.

Eggs, excreta, and tissues were homogenized, combusted and analyzed by LSC for radioactivity content.

Fat and skin samples were homogenized, digested in scintillation cocktail and then analyzed directly by LSC.

All tissue and egg samples were extracted 2-3x sequentially with acetone, methanol and methanol:water (1:1); the individual extracts were combined. Fractions containing the highest concentration of radioactivity (found by LSC) were analyzed by TLC and HPLC to identify the major metabolites in meat, tissue and eggs.

Liver non-extractable samples were enzyme-treated with protease, β-glucoronidase, or sulfatase. After centrifugation, the pellet was extracted by methanol. The supernatant was analyzed by TLC. Samples of liver non-extractable, egg yolk non-extractable, supernatant of protease treated liver non-extractable, reference standard (N-acetyl cysteine AMPA) and extracts of egg yolk and excreta acetone fractions were treated with acid (HCl) in an attempt to characterize non-extractable radioactivity and unknown polar components detected in extracts. Samples were extracted with methanol, acetone or ethyl acetate and analyzed by TLC and HPLC. MS was used to obtain spectral data on the isolated excreta metabolites.

Acetone extractable radioactive residues from samples of excreta, liver, kidney, muscle, fat, egg yolk and egg white were analyzed using 2D-TLC and HPLC to determine the metabolic profile in each sample. Metabolites were identified by comparing reference standards to the examined extracts.

Metabolites present in the excreta were investigated to aid in the identification or characterization of metabolites found in tissues. Isolation of polar metabolites from excreta was conducted by initially extracting homogenized excreta in acetone.

After purification, the resulting residue was dissolved in HPLC initial mobile phase and analysed by HPLC. Fractions with the most ^{14}C-metabolites were combined, concentrated and subjected to strong cation exchange HPLC-MS analysis.

Radio-analyses were validated by analysing triplicate aliquots each of control excreta, egg yolk and representative tissues that were fortified with a known amount of ^{14}C-fluazinam. Overall mean recovery was 102%. Therefore the measured dpm values were not corrected for recovery. The limit of detection by LSC was calculated to be 0.0007 to 0.0016 mg-equ/kg for the phenyl-label and 0.0006 to 0.0016 mg-equ/kg for the pyridyl-label, related to a representative sample aliquot weight of 0.2 to 0.5 g.

Findings:

The majority of administered radioactivity was recovered in the excreta (including the GI tract and the pan wash and wipe): 113% and 111% of the administered radioactivity was excreted (group 2 and 3, respectively). The entire egg production contained less than 0.65% of the dose for each label. Among edible tissue, the highest residue levels were found in the liver and abdominal fat, followed by skin with fat, kidneys and muscle. These contained less than 2.4% of the total dose. The detailed values are listed in Table B.7.2.2-1:

Table 7.2.2-1: Radioactivity levels in samples from laying hens after oral administration of[14C]IKF-1216 (fluazinam):

Sample	Phenyl label (group 2)		Pyridyl label (group 3)	
	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose
Blood	0.392	0.14	0.215	0.08
Fat (abdominal)	0.936	0.57	0.959	0.49
Kidneys	0.438	0.11	0.349	0.09
Liver	1.047	0.92	0.920	0.88
Muscle (breast)	0.026	0.08	0.021	0.06
Muscle (thigh)	0.059	0.11	0.047	0.08
Skin with fat	0.493	0.45	0.581	0.58
Egg White	0.040	0.04	0.039	0.03
Egg Yolk	1.169	0.52	1.022	0.35
Total in Tissues and Eggs	*	2.94	*	2.64
Excreta	*	101	*	99.1
Pan paper wash	*	0.70	*	0.49
GI tract and contents	*	11.1	*	11.9
Total	*	**116**	*	**114**

* Not reported.

There was apparently no difference in elimination and distribution of radioactivity between samples from hens administered the phenyl-labelled or pyridyl-labelled ^{14}C-fluazinam, indicating that the aryl-amine-pyridyl bond was intact.

The distribution of radioactivity in eggs as a function of time is shown in Table 7.2.2-2. Radioactivity was incorporated into the eggs beginning with the collection on day 3. As would be expected due to the study length, residue levels in eggs did not appear to reach a plateau level by the end of the study.

Table 7.2.2-2: Daily radioactivity concentrations in eggs from laying hens after oral administration of[14C]IKF-1216 (fluazinam):

Collection time	Phenyl label (group 2)				Pyridyl label (group 3)			
	Egg whites		Egg yolks		Egg whites		Egg yolks	
	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose	[mg-equ/kg]	% of administered dose
Day 1	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *
Day 2	0.003	<0.01	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *	n.d. *
Day 3	0.016	<0.01	0.154	0.03	0.016	0.01	0.162	0.06
Day 4	0.027	0.02	0.598	0.19	0.029	0.01	0.680	0.13
Sacrifice (Day 4)	0.040	0.02	1.169	0.30	0.039	0.01	1.022	0.16
Total	0.086	0.04	1.921	0.52	0.084	0.03	1.864	0.35

* n.d.: not detected

The radioactivity was separated into aqueous extractable, organic extractable and non-extractable solid fractions. The breakdown of each matrix into these fractions is shown in Table 7.2.2-3.

Table 7.2.2-3: Distribution of radioactivity in extractable and non-extractable fractions in samples from laying hens after oral administration of[14C]IKF-1216 (fluazinam):

Sample	Acetone extractable [1]		Methanol extractable		MeOH/Water extractable		Non-extractable solid		Total
	ppm [2]	% TRR	ppm [2]	% TRR	ppm [2]	% TRR	ppm [2]	% TRR	[%]
Phenyl label (group 2)									
Liver	0.50	48.1	0.04	3.7	0.02	1.8	0.46	44.0	97.6
Kidney	0.27	62.0	0.02	5.4	<0.01	2.1	0.13	28.5	98.0
Muscle	0.04	59.6	<0.01	6.9	<0.01	1.3	0.02	31.4	99.2
Fat	1.04	111.5	0.01	1.1	<0.01	0.1	<0.01	0.5	113.2
Egg yolk	0.86	73.7	0.03	2.4	<0.01	0.8	0.25	21.1	98.0
Egg white	0.04	92.7	<0.01	5.1	<0.01	3.8	<0.01	7.6	109.2

Sample	Acetone extractable [1]		Methanol extractable		MeOH/Water extractable		Non-extractable solid		Total
	ppm [2]	% TRR	ppm [2]	% TRR	ppm [2]	% TRR	ppm [2]	% TRR	[%]
Excreta [3]	0.50	37.3	0.04	7.8	0.02	9.5	0.46	-	-
Pyridyl label (group 3)									
Liver	0.44	48.1	0.04	3.8	0.02	1.7	0.49	52.8	106.4
Kidney	0.22	63.4	0.02	5.5	<0.01	1.9	0.11	32.6	103.0
Muscle	0.03	57.4	<0.01	8.4	<0.01	2.2	0.02	32.1	100.1
Fat	0.97	100.9	0.01	1.1	<0.01	0.1	<0.01	0.4	102.5
Egg yolk	0.76	74.7	0.03	3.2	<0.01	0.8	0.24	23.7	102.4
Egg white	0.04	93.1	<0.01	4.4	<0.01	2.2	<0.01	3.4	103.1
Excreta [3]	0.44	40.0	0.04	7.1	0.02	8.8	0.49	-	-

1 sum of two extractions (single extraction for liver and excreta)

2 ppm is equal to mg-equ/kg

3 Excreta non extractable solid was not further characterised

Two-dimensional TLC and HPLC analyses revealed several components. Similar profiles were found among the examined tissue extracts. Five components with low polarity were confirmed: Fluazinam, MAPA, DAPA, AMPA and HYPA: Fluazinam was detected only in small amounts with total radioactivity less than 3.0%. The major metabolite in muscle, fat, liver, egg white and egg yolk was identified to be AMPA, the product of reduction of fluazinam. It accounted for 6.06% (0.071 mg-equ/kg) to 81.9% (0.767 mg-equ/kg) of total radioactivity in the examined samples. The reduction products MAPA and DAPA were also detected in all examined samples with combined amounts ranging from 2.71% (0.032 mg-equ/kg) to 14.7% (0.138 mg-equ/kg). The dehalogenated/hydroxylated metabolite HYPA was also identified.

A group of polar metabolites (10, 11, 12, 13) was detected at low levels. These four unknowns from liver and eggs corresponded to conjugates resulting from replacement of the phenyl ring chlorine atom by glutathione and therefore were considered to be N-acetyl cysteine conjugates of AMPA and fluazinam. These conjugates cannot be converted back to AMPA or fluazinam because their formation resulted from replacement of the chlorine atom by an amino acid substituents attached through a carbon-sulfur covalent bond. The glutathione substituents underwent further metabolism by a complex series of reactions.

Another group of less polar metabolites (5, 6, 8, 9) was detected at low levels.
When control kidney sample was fortified with 14C-fluazinam, one polar component (14) was detected in addition to fluazinam.
The results of the metabolite analyses are summarized in Tables 7.2.2-4 and 7.2.2-5.

Table 7.2.2-4: Metabolites [%TRR] detected in samples from laying hens after oral administration of[14C]IKF-1216 (fluazinam):

Metabolite	Liver	Kidney	Muscle	Fat	Egg White	Egg Yolk
Phenyl label (Group 2)						
Fluazinam	2.74	<1.00	1.13	2.21	<1.00	1.53
MAPA	2.50	1.61	2.50	8.84	3.43	1.46
DAPA	3.17	1.98	6.02	5.90	4.54	1.25
AMPA	13.1	18.0	32.4	81.9	48.5	6.06
5	2.16	3.29	2.09	2.95	6.21	1.25
6	1.39	3.35	n.d. *	n.d. *	17.4	1.67
HYPA	4.86	3.16	5.60	2.63	2.50	5.30
8	1.97	2.48	1.55	n.d. *	1.95	2.96
9	1.54	2.54	1.67	<1.00	1.67	4.53
10	5.87	10.1	1.43	<1.00	4.45	6.27
11	2.74	4.28	3.22	<1.00	<1.00	11.2
12	4.79	8.87	0.66	<1.00	<1.00	2.79
13	<1.00	<1.00	1.31	<1.00	n.d. *	20.0
14	1.64	1.55	n.d. *	<1.00	<1.00	1.95
Total	**48.4**	**61.2**	**60.6**	**104**	**90.7**	**68.2**
Pyridyl label (Group 3)						
Fluazinam	2.65	1.64	<1.00	2.10	<1.00	<1.00
MAPA	2.16	2.77	2.35	7.54	3.07	1.55
DAPA	2.50	3.15	6.20	6.21	7.54	2.83
AMPA	13.8	19.0	30.1	67.9	43.4	12.4
5	2.74	3.28	1.21	1.81	9.12	<1.00
6	2.41	3.40	n.d. *	n.d. *	19.1	1.27
HYPA	4.95	3.84	5.91	4.30	2.79	3.81
8	2.12	2.08	1.84	1.53	1.58	4.09
9	0.91	1.70	2.70	2.29	1.68	3.95
10	5.44	10.5	3.56	1.53	2.98	8.39
11	2.98	3.28	<1.00	<1.00	<1.00	9.02
12	3.27	6.24	1.44	<1.00	n.d. *	1.41
13	<1.00	<1.00	<1.00	n.d. *	n.d. *	17.6
14	1.73	1.64	<1.00	n.d. *	<1.00	2.40
Total	**47.7**	**59.5**	**55.3**	**95.2**	**91.3**	**68.7**

* n.d.: not detected

Table 7.2.2-5: Metabolites [mg-equ/kg] detected in samples from laying hens after oral administration of[14C]IKF-1216 (fluazinam):

Metabolite	Liver	Kidney	Muscle	Fat	Egg White	Egg Yolk
Phenyl label (Group 2)						
Fluazinam	0.027	<0.01	<0.01	0.020	<0.01	0.018
MAPA	0.024	<0.01	<0.01	0.079	<0.01	0.018
DAPA	0.031	<0.01	<0.01	0.055	<0.01	0.015
AMPA	0.127	0.070	0.019	0.767	0.019	0.071
5	0.021	0.013	<0.01	0.027	<0.01	0.015
6	0.014	0.013	n.d. *	n.d. *	<0.01	0.020
HYPA	0.048	0.012	<0.01	0.024	<0.01	0.062
8	0.015	0.010	<0.01	n.d. *	<0.01	0.036
9	0.015	0.010	<0.01	<0.01	<0.01	0.053
10	0.057	0.039	<0.01	<0.01	<0.01	0.074
11	0.027	0.017	<0.01	<0.01	<0.01	0.131
12	0.040	0.035	<0.01	<0.01	<0.01	0.033
13	<0.01	<0.01	<0.01	<0.01	n.d. *	0.234
14	0.016	<0.01	n.d. *	<0.01	<0.01	0.023
Pyridyl label (Group 3)						
Fluazinam	0.022	<0.01	<0.01	0.020	<0.01	<0.01
MAPA	0.018	<0.01	<0.01	0.072	<0.01	0.016
DAPA	0.021	<0.01	<0.01	0.059	<0.01	0.031
AMPA	0.115	0.055	0.014	0.651	0.017	0.127
5	0.023	<0.01	<0.01	0.017	<0.01	<0.01
6	0.020	0.010	n.d. *	n.d. *	<0.01	0.013
HYPA	0.041	0.011	<0.01	0.041	<0.01	0.039
8	0.017	<0.01	<0.01	0.014	<0.01	0.042
9	<0.01	<0.01	<0.01	0.022	<0.01	0.040
10	0.045	0.030	<0.01	0.014	<0.01	0.088
11	0.025	<0.01	<0.01	<0.01	<0.01	0.092
12	0.027	0.018	<0.01	<0.01	n.d. *	0.014
13	<0.01	<0.01	<0.01	n.d. *	n.d. *	0.180
14	0.014	<0.01	<0.01	n.d. *	<0.01	0.025

* n.d.: not detected

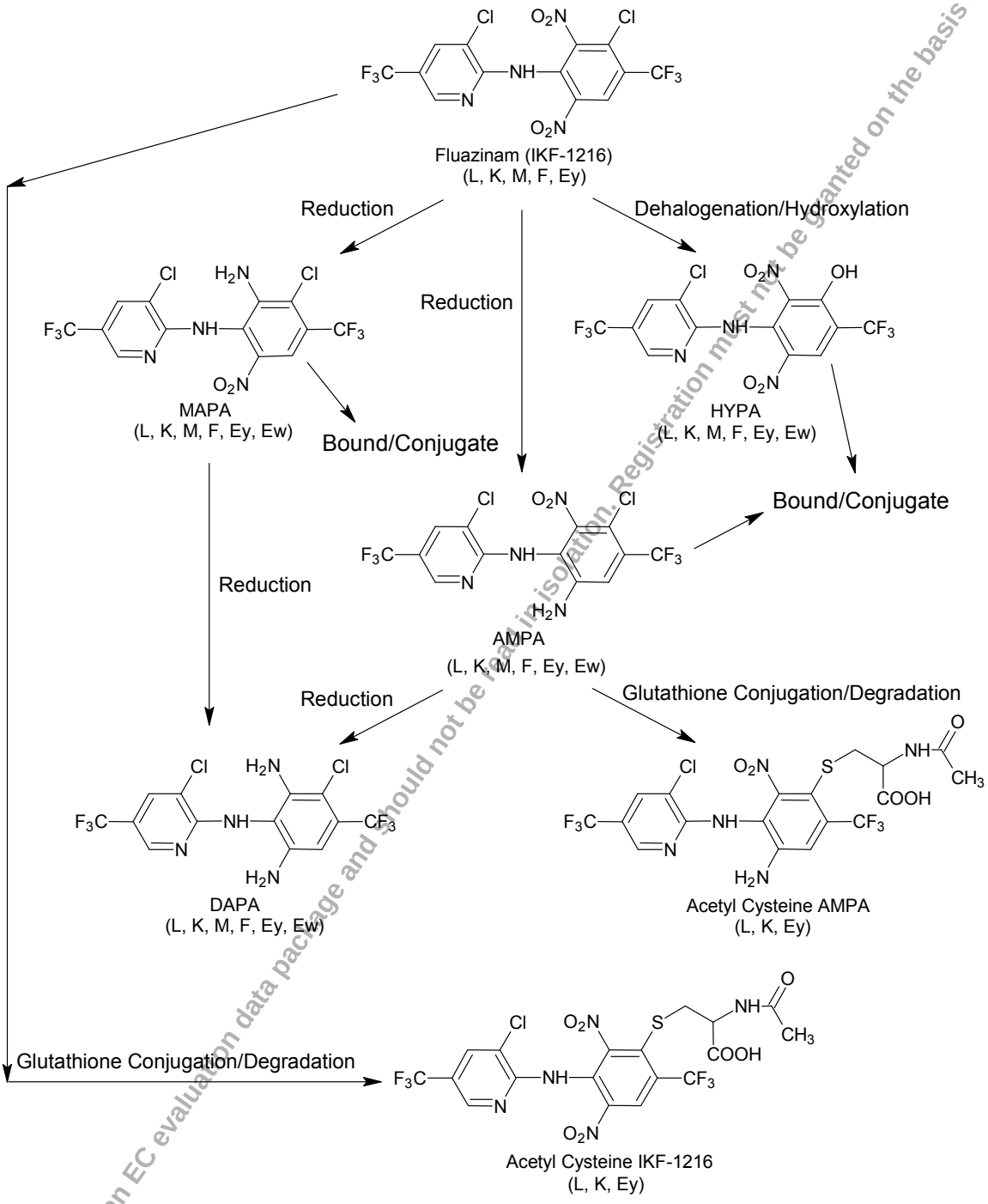
Enzymatic treatment of the liver non-extractable fraction with protease released approximately half of the bound radioactivity into the aqueous fraction, indicating that at least this portion of the

bound radioactivity was associated with peptides or proteins. Subsequent treatment of the sample with acid released AMPA, suggesting that the liver bound fraction consisted largely of peptide or protein bound AMPA. Besides, also a small quantity of one less polar product was released which was identified as the acid re-arrangement product of AMPA. Treatment of the liver non-extractable fraction with either sulfatase or β-glucuronidase did not release any of the bound radioactivity.

Acid hydrolysis of the liver non-extractable fraction released all of the bound radioactivity through use of increasing acid strength (successive treatment with 1N, 6N and 12N HCl). The major identifiable component was AMPA, with a lesser amount of the AMPA re-arrangement isomer (identified in the goat metabolism study, Report No: 5248-92-0116-EF) also present. Other components were very polar; they have been tentatively assigned as glutathione conjugates based on similar chromatographic behaviour to the polar components detected in the methanol extract of excreta samples.

The metabolic pathway of ^{14}C-fluazinam in laying hens is proposed in the figure below:

Fig. 7.2.2-6: Proposed metabolic pathway of fluazinam in laying hens:



(Letters in parentheses indicate matrix in which metabolite was identified:
L=Liver, K=Kidney, M=Muscle, F=Fat, Ey=Egg yolk, Ew=Egg white)

Conclusion:

In laying hens, the major metabolites were reduction products, primarily AMPA, DAPA, MAPA and HYPA, as well as measurable amounts of fluazinam parent molecule. Other metabolites found also included a mixture of conjugates representing the products of glutathione conjugation and subsequent metabolism. A summary of the combined residue data for hens dosed with

fluazinam is presented in Table 7.2.2-7.

Table 7.2.2-7: Summary of residue data for laying hens fed with [14C]IKF-1216 (fluazinam) at 0.76 mg/kg bw/d diet level 1

	Muscle (Dark)[1]	Eggs[2]	Fat	Liver
TRR[3] (mg/kg)	0.053	0.388	0.948	0.984
Extractable (mg/kg)	0.036	0.306	0.944	0.517
Non-extractable (mg/kg)	0.017	0.082	0.004	0.467
Fluazinam (mg/kg)	<0.01	<0.01	0.02	0.03
AMPA (mg/kg)	0.02	0.05	0.71	0.12
DAPA (mg/kg)	<0.01	0.01	0.06	0.03
MAPA (mg/kg)	<0.01	<0.01	0.08	0.02
HYPA (mg/kg)	<0.01	0.02	0.03	0.04
Unknowns[4] Number	0	11	6	11

1 Residue characterized only for dark muscle; light muscle had 0.024 mg/kg TRR

2 Whole egg residues calculated from data for egg whites and egg yolks based on yolk comprising 33% of egg and whites 67% of egg by weight

3 Values in table are averages for phenyl and pyridyl labels

4 Unknowns included a mixture of glutathione derived conjugates of AMPA and fluazinam. Columns list ≥0.01 mg/kg; number observed and mg/kg equivalents as range.

As residue levels in eggs did not appear to reach a plateau level by the end of the study, theoretical maximum transfer factors were not estimated for laying hen.

B.7.3 Definition of the residue (Annex IIA 6.7; Annex IIIA 8.6)

For definition of the relevant residues of fluazinam in raw agricultural commodities the following facts were taken into account:

Based on the chemical composition of identifiable residues in the metabolism study in potatoes (on which the use of fluazinam is intended), as well as on the supporting metabolism studies on peanut, apple and grape metabolism, the residue in these crops can be defined by quantification of the parent molecule, fluazinam (IKF-1216). The phase 1 of the metabolic pathway of fluazinam in plants involves reduction of one or both nitro groups (to form AMPA or DAPA), replacement of the phenyl ring chlorine by glutathione conjugation (forming AMGT in plants) and then further metabolism or conjugation. This general pathway for formation of phase I metabolites is the same in animals and plants.

Parent fluazinam is rapidly degraded and is either **not found** or barely detectable **in peanuts and potatoes**. Fluazinam parent was the **major identifiable residue in** a **grape** metabolism study. Identifiable residues in very low amounts either closely resemble fluazinam in structure or are the result of re-incorporation of the fluazinam carbon pool into natural products.

The residue in the supported crop can be defined only by quantification of the parent molecule fluazinam.
Therefore the proposed residue definition in plants for risk assessment and monitoring purposes is **fluazinam**.

Metabolism studies in livestock showed only little transfer of fluazinam into edible tissues, milk and eggs. No accumulation of the active substance was observed in livestock animals. As only low residues of fluazinam are expected in potential feeding stuffs (<0.01 mg/kg in potatoes), a residue definition for food of animal origin is not proposed.

B.7.4 Use pattern

Fluazinam-containing formulations are used within the European Union. Based on the data submitted by the notifier they are registered in 13 EU Member States for field use on potatoes to control the harmful organism *Phytophtora infestans*.

The individual member states' uses are summarized in table B.7.4-1 reflecting the status of registration submitted by the notifier.

Table B.7.4-1 Use pattern of existing authorized uses of fluazinam in the EU Member States.

Country	Application rate per treatment [L product/ha]	Number of applications	Spray interval in days	Time of application (growth stage)	PHI [days]
Austria	0.4	1-10	10-14 (low risk), 7-10 (high risk)	First blight warning or BBCH 35	7
Belgium	0.3 0.4	1-10	7 (low risk) 10 (high risk)	First blight warning or BBCH 35	7
Denmark	0.2-0.4	1-8	7-10	First blight warning or BBCH 35	7
Finland	0.4	Max. 4 per year	7-14	First blight warning or BBCH 35	7
France	0.4	Not specified	7	First blight warning or BBCH 35	7
Germany	0.4	Max. 8	7	First blight warning or BBCH 35	7
Greece	0.3-0.4	Max. 6	7	When weather conditions favour disease development or at 15-20cm plant height	7
Ireland	0.4	5-7 for severe, 7-10 for high risk situations	7-10	First blight warning or BBCH 35	0
Italy	0.3-0.5	Not specified	6-10	First blight warning or BBCH 35	7

Country	Application rate per treatment [L product/ha]	Number of applications	Spray interval in days	Time of application (growth stage)	PHI [days]
Netherlands	0.4	Not specified	7-10	First blight warning or BBCH 35	0
Portugal	0.3-0.4	Max. 10	Max. 14	First blight warning or BBCH 35	7
Sweden *	0.3-0.4	3-8	7-10	First blight warning or BBCH 35	7-10
United Kingdom	0.3	10 per crop	5-7 for severe, 7-10 for high risk situations	First blight warning or BBCH 35	0

* Also authorized with some effect on *Botrytis* and *Slecrotinia sclorotiorum*

In contrast to the individual member states' GAPs listed above, the intended uses of plant protection products containing fluazinam, to which residue trial data have been submitted by the notifier, are listed in table B.7.4-2.

Table B.7.4-2 Intended uses of fluazinam i.e. supported uses for evaluation under Directive 91/414/EEC.

Crop	Country	Use	Concentration of a.i. in the formulation	Application rate per treatment **[L product/ha]**	Application rate per treatment **[kg a.i./ha]**	Number of applica-tions	Interval between applica-tions (days)	Time of applica-tion (growth stage)	PHI [days]
Potatoes	EU	field	500 g/L	0.4	0.2	Max. 10	7	BBCH 95-97 *	7

* Last application

B.7.5 Identification of critical GAPs

More critical GAPs currently authorised in the EU Member States are summarised in table B.7.5-1. However, the evaluation in this report is done for the intended use supported by the data provided by the notifier.

Table B.7.5-1 Critical GAPs.

Country	Application rate per treatment [L product/ha]	Number of applications	Spray interval in days	Time of application (growth stage)	PHI [days]
Ireland	0.4	5-7 for severe, 7-10 for high risk situations	7-10	First blight warning or BBCH 35	0
Netherlands	0.4	Not specified	7-10	First blight warning or BBCH 35	0
United Kingdom	0.3	10 per crop	5-7 for severe, 7-10 for high risk situations	First blight warning or BBCH 35	0

B.7.6 Residues resulting from supervised trials (Annex IIA 6.3; Annex IIIA 8.2)

Fluazinam is supported for use in **potatoes**.

The critical GAP for Northern Europe for fluazinam on potatoes is: 200 g ai/ha, 10 treatments at 7 days spray interval and a PHI of 7 days. The GAP for Southern Europe for fluazinam on potatoes is: 200g ai/ha, 6 treatments at 7 days spray interval and a PHI of 7 days.

In total 13 trials have been performed at the critical Northern European GAP ± 25%. The number of applications ranged from 8 to 11 and the PHI ranged from 6 – 7 days. These trials were conducted in Germany (8) and the UK (5) during 1989 – 1996. Within the different residue trials for potatoes **decline/degradation studies** have been carried out as follows:

Sampling points (PHI): 0, 7, 14 days = 4 trials
Sampling points (PHI): 0, 7 days = 3 trials
Sampling points (PHI): 0, 6, 13 days = 1 trial

Fluazinam residues were below the LOQ at all sampling points in these decline studies.

The trials conducted in Southern Europe (Greece) used a PHI of 14 - 15 days and included 6 applications. The trials spanned a two year period. Although the PHI in the trials was not at the GAP, the residue decline studies described above demonstrate that PHI is not an issue for fluazinam residues. This consideration can be supported by the results of the plant metabolism study in potatoes (see B.7.1.1: Metabolism in potatoes).

In all 16 trials, no residues of fluazinam at or above the limit of quantification of the method (0.01 mg/kg) were found in the tubers.

Method:

Samples were prepared using a homogeniser, until a completely homogenous sample was obtained. Samples were stored frozen until analysis; the corresponding storage periods are all covered by the tested storage stability for fluazinam in potatoes (26 months; basing on the results of the study by: Ryan, J., Sapiets, A. (1993): Fluazinam: Storage Stability of the Residues in Frozen Crop Samples, point B.7.6.3.2).

The analytical method used for the residue studies from the northern zone involved extraction with methanol, sub-sampling of an aliquot and dilution of the aliquot with a pH 7-buffer solution. The aqueous samples were partitioned into dichloromethane, then cleaned up by adsorption chromatography on a silica cartridge. Final determination was by gas chromatography with electron capture detection (in one report the final determination was by HPLC with UV-detection).

The analytical method used for the residue studies from the southern zone involved extraction with methanol/acetic acid. An aliquot of the filtered extract was acidified with 0.2 N HCl and partitioned with hexane. The hexane phase was then partitioned with 0.5 N NaOH solution. The aqueous phase was acidified with concentrated HCl and partitioned with hexane. The hexane was then evaporated. The sample was diluted with hexane and passed through a clean-up column prior to gas chromatographic quantitation with electron capture detector.

Both methods provided a LOQ of 0.01 mg/kg and showed satisfactory recoveries.

The results of analysis are summarised in table B.7.6-1 for fluazinam; the parent compound was

provisionally defined as the relevant residue. For more detailed data see “Annex Ia: Summary of trial information – Potatoes”.

Table B.7.6-1 Overview of European residue data for fluazinam in potatoes:

Crop, Region, Countries (no. of trials), year	Application			Residue		Reference
	Formulation content of a.s.	Rate [kg a.s./ha]	Number of applications.	DAT [days]	fluazinam [mg/kg]	
Potatoes Germany (4) 1990	SC 500 g/L	0.200	8-10	0 7 14	<0.01 <0.01 <0.01	Ryan J., Sapiets A. 1991 Report Nr. RJ1023B GLP: Yes
Potatoes Germany (4) 1989	SC 500 g/L	0.200	8-10	0 7 14	<0.01 <0.01 <0.01	Ryan J., Sapiets A. 1991 Report Nr. RJ1025B GLP: Yes
Potatoes United Kingdom (2) 1996	SC 500 g/L	0.200	9-10	7	<0.01	Clarke D.M. 1997 Report Nr. RJ2308B GLP: Yes
Potatoes United Kingdom (3) 1989	SC 500 g/L	0.200	8-11	7	<0.01	Burke S.R., Sapiets A. 1992 Report Nr. RJ1161B GLP: Yes
Potatoes Greece (1) 1995	SC 500 g/L	0.200	6	15	<0.01	Dvorak R.S., Kenyon R.G.; 1996 Report Nr. 6530-96-0020-CR-001 GLP: Yes
Potatoes Greece (2) 1996	SC 500 g/L	0.250	6	14	<0.01	Kenyon R.G. 1998 Report Nr. 6953-97-0087-CR-001 GLP: Yes

Remark of RMS:

Additional residue trials conducted in Northern Europe were submitted by the notifier using a range of applications (4-12) combined with application rates from 150 to 500 g fluazinam/ha and PHIs ranging from 7 to 58 days. Although the residues in the tubers were all <0.01 mg/kg the study design of these trials does not represent the supported GAP. Therefore they were not included in the evaluation; however references are listed below in table B.7.6-2:

Table B.7.6-2 References of additionally provided residue trials in potatoes not representing the supported GAP:

Crop, Region, Countries (no. of trials), year	Application			Residue		Reference
	Formulation content of a.s.	Rate [kg a.s./ha]	Number of applications.	DAT [days]	fluazinam [mg/kg]	
Potatoes France, North, (6) 1991	SC 500 g/L	0.150 0.200 0.250 0.150 0.200 0.250	8 8 8 8 8 8	34 34 34 44 44 44	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	Ryan J., Sapiets A. 1992 Report Nr. RJ1105B GLP: Yes
Potatoes France, North, (6) 1991	SC 500 g/L	0.150 0.200 0.250 0.150 0.200 0.250	7 7 7 11 11 11	57 57 57 31 31 31	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01	Ryan J., Sapiets A. 1992 Report Nr. RJ1106B GLP: Yes
Potatoes France, North, (5) 1990	SC 500 g/L	0.250 0.350 0.500 0.500 0.375	4 4 7 3 6	10 10 27 29 35	<0.01 <0.01 <0.01 <0.01 <0.01	Schulz H. 1992 Report Nr. RCC project 284670, FZM/SOLTU 12/F/90 GLP: Yes
Crop, Region, Countries (no. of trials), year	Application			Residue		Application
	Formulation content of a.s.	Rate [kg a.s./ha]	Number of applications.	DAT [days]	[mg/kg]	
Potatoes France, North, (5) 1990	SC 500 g/L				**MAPA**	Mirbach M.J., Ullrich A.; 1991 Report Nr. RCC project 284670, FZM/SOLTU 11/F/90 GLP: Yes
		0.250 0.350 0.500 0.500 0.375	4 4 7 3 6	10 10 27 29 35	<0.01 <0.01 <0.01 <0.01 <0.01	
					HYPA	
		0.250 0.350 0.500 0.500 0.375	4 4 7 3 6	10 10 27 29 35	<0.01 <0.01 <0.01 <0.01 <0.01	

Conclusion:

In 16 residue field trials no residues at or above the LOQ of 0.01 mg/kg have been determined in the potato tubers at harvest. This is consistent with the results obtained in the potato plant metabolism study.

Within the different residue trials for potatoes several decline/degradation studies have been carried out. Fluazinam residues were below the LOQ at all sampling points (PHI 0, 6/7 and 13/14

days) in the residue decline studies.

Only three trials were available for Southern Europe. These trials were conducted in Greece; using a PHI of 14 - 15 days and including 6 applications. The trials spanned a two year period. Although the PHI in the trials was not at the GAP, the lack of residues even at 0 day PHI intervals observed in the residue decline studies described above as well as the results of the plant metabolism study in potatoes demonstrate that no residues of fluazinam above the LOQ of 0.01 mg/kg in potatoes should be expected in any planting situation.

The thirteen Northern European trials were conducted over more than one season and were performed at the critical Northern European GAP or within 25% of it. These data are sufficient to propose an EU MRL. Fluazinam, when applied to potatoes according to GAP, does not lead to residues in potato tubers at or above the limit of quantification of 0.01 mg/kg; **an EU MRL of 0.01 mg/kg (LOQ) can be proposed.**

B.7.6.1 Overall conclusion

Residue trials with fluazinam were performed in a representative selection of regions and locations in Europe. A suspension concentrate formulation was used applying fluazinam to usual practice. The rates in the residue trials from northern Europe corresponded to the maximum seasonal dose rate applied for the active substance. The trials therefore demonstrated the typical residue behaviour of fluazinam in potatoes under European conditions. Residue trials from southern Europe were provided in a limited number (3 trials) and a prolonged pre-harvest interval (15 days). These deficiencies can be amended by the general outcome of the plant metabolism study on potatoes and eight residue decline studies in potatoes, showing a zero residue-situation in potato tubers from the day of the last application up to day 14 after last application.

B.7.6.2 Stability of residues prior to analysis

B.7.6.2.1 Storage stability in potatoes and potato processed fractions

Reference:

McFall, D.D. (1999): Stability of Fluazinam in Potatoes and Potato Processed Fractions after Freezer Storage; (Report No: 6166-94-0148-CR-002).

Guidelines: EPA Pesticide Assessment Guidelines, Subdivision O, Section 171-4(e), OPPTS Guideline 860.1380.

GLP: Yes.

Study considered acceptable.

Material and Methods:

The study was conducted using potatoes and processed fractions from local or commercial sources. Whole potato tubers were chopped and homogenized using a Hobart food chopper. Locally purchased potato chips were crushed in the bag and mixed by hand. Potato granules and wet peels, obtained from a processor, were thoroughly mixed prior to weighing of stability samples. Other than mixing, no further sample preparation was necessary for these matrices. Due to the processing samples of chips and granules were presumed to be homogenous; where

two different lots of a processed fraction were used, they were thoroughly mixed. From the composed samples, 10 g samples were weighed at room temperature into appropriate bottles fitted with screw caps; the same sample size was used for the processed fractions. Stability samples (four of seven crop aliquots) were fortified in the bottles at 0.50 mg/kg by addition of fluazinam in a suitable solvent. The other three crop aliquots were prepared and stored to run one control and two concurrent procedural recovery samples, fortified with fluazinam on the day of analysis. Residues of fluazinam were extracted from potato fractions by blending with methanol and acetic acid. An aliquot of the filtrate was acidified with 0.2 N HCl and partitioned with hexane. The hexane phase was then partitioned with 0.5 N NaOH, and the hexane discarded. The remaining aqueous phase was acidified and again partitioned with hexane. The organic phase was concentrated by rotary evaporation and analysed by GC using an electron capture detector. If matrix interferences complicated the determination, an optional column cleanup was incorporated.

The procedure was validated prior to initiation of the study as well as with recovery samples analysed concurrently with each set of stored samples. Aliquots of the potato fractions were amended prior to extraction by the addition of fluazinam at a level of 0.50 mg/kg. The limit of determination was 0.025 mg/kg for 10 g-samples. Concurrent control samples were analysed to calculate net recovery values for the concurrent recovery samples. The results reflecting the procedural recovery for fluazinam in each matrix are summarised in table B.7.6.2.1-1:

Table B.7.6.2.1-1 procedural recovery data for fluazinam in each potato matrix from concurrent stability samples fortified at 0.50 mg fluazinam/kg.

Matrix	Range of recoveries [%]	Mean recovery [%]	Standard deviation
Potatoes	96 – 115	107	7
Wet potato peels	83 – 117	97	12
Potato chips	73 – 114	90	14
Potato granules	89 - 119	105	9

Based on the data shown in table B.7.6.2.1-1 it can be concluded that the analytical method applied is acceptable.

Findings:

The uncorrected mean recoveries (mean value of 4 samples) from each sampling interval are shown in table B.7.6.2.1-2 for all potato matrix samples fortified with fluazinam (0.50 mg/kg) and kept frozen over a three year storage period.

Table B.7.6.2.1-2 Mean recoveries of fluazinam from at 0.50 mg fluazinam/kg fortified potato matrices after three years freezer storage and the corresponding % decline.

Storage interval *	Whole potatoes		Wet potato peels		Potato chips		Potato granules	
	[mg/kg]	% decline **	[mg/kg]	% decline **	[mg/kg]	% decline **	[mg/kg]	% decline **
0 days	0.54	---	0.41	---	0.49	---	0.59	---

Storage interval *	Whole potatoes		Wet potato peels		Potato chips		Potato granules	
	[mg/kg]	% decline **	[mg/kg]	% decline **	[mg/kg]	% decline **	[mg/kg]	% decline **
1 days	0.48	**11**	0.44	**---**	0.46	**6**	0.46	**22**
21 days	0.49	**9**	0.38	**7**	0.42	**14**	0.52	**12**
49 days	0.45	**17**	0.40	**2**	0.45	**8**	0.33	**44**
3 months	0.43	**20**	0.34	**17**	0.38	**22**	0.32	**46**
6 months	0.51	**6**	0.37	**10**	0.43	**12**	0.24	**59**
12 months	0.40	**26**	0.36	**12**	0.34	**31**	0.31	**47**
18 months	0.36	**33**	0.31	**24**	0.34	**31**	0.15	**75**
24 months	0.35	**35**	0.35	**15**	0.42	**14**	0.26	**56**
30 months	0.30	**44**	0.30	**27**	0.33	**33**	0.27	**54**
36 months	0.31	**43**	0.29	**29**	0.37	**24**	0.18	**69**

* For simplifying the data representation and a better overview the varying day-intervals of storage among the different fractions were pooled in months.

** Significant degradation is defined in the EU guidelines (Doc 7032/VI/95 rev. 5, dated July 27, 1997) as >30% over the storage period.

The reported declines are simply the percent differences between the 0-day observations and the last observations in the series. Fluazinam, amended to potato RAC and processed fractions (chips, granules and wet peels) was shown to decline during frozen storage in all matrices. These declines were 43 % for potatoes, 24 % for potato chips, 69 % for potato granules and 29 % for wet potato peels after 36 months of frozen storage.

Conclusion:

Based on the fortification concentration of 0.50 mg fluazinam/kg, whole potato homogenates were shown to be stable up through 1 year; potato chips and wet peels were stable through the entire storage period (3 years). Only fluazinam values in potato granules showed a dramatically decline and were stable only up to 21 days.

Remark of RMS:

The study was considered valid; however, the fortification level of 0.50 mg fluazinam/kg is too high to be representative for the residue situation in the supported crop. The fortification level of 0.10 mg fluazinam/kg used in the crop storage stability study by Ryan, J., Sapiets, A. (1993), point B.7.6.2.2, is certainly more appropriate to point out a realistic behaviour of fluazinam residues under storage conditions.

B.7.6.2.2 Crop storage stability

Reference:

Ryan, J., Sapiets, A. (1993): Fluazinam: Storage Stability of the Residues in Frozen Crop Samples. -Final Report. ; (Report No: RJ1583B).

Guidelines: None stated, but conforms to EC Commission Document 7032/VI/95 – Rev.5, Storage Stability of Residue Samples. Deviation: Storage was run under refrigeration at <-15 °C instead of <-18 °C.

GLP: Yes.

Study considered acceptable, taking into account the outcome of the study.

Material and Methods:

Untreated coffee, potato, onion and grape (fruit and wine) samples were previously analysed and found to contain no measurable residues of fluazinam. Two samples of each crop type were bulked and homogenised prior to removing individual sub-samples for the study.

Aliquot samples of homogenised crops (20 g) were weighed into appropriate tubs and fortified with fluazinam each at 0.1 mg/kg, wine samples (50 mL) at 50 µg/L, by the addition of a fluazinam standard solution in acetone. The sample tubs were sealed and stored in a temperature monitored freezer at <-15 °C for up to 26 months. At intervals, duplicate samples were removed from the freezer and analysed. After extraction, the methanol extracts were stored in a cold room at <8 °C for various periods prior to analysis.

The samples were extracted with methanol, filtered and the filtrate adjusted to a known volume with further solvent. Depending on the substrate to be analysed, either an aliquot was diluted with pH 7 buffer and the analyte partitioned into dichloromethane, or an aliquot was evaporated to dryness and cleaned up by liquid-liquid partition chromatography. After concentration the analyte was cleaned up on silica cartridges. Final quantitative determination was by gas-liquid chromatography wit electron-capture detection. In a few instances, co-extractives could not be separated from the peak of interest, so the samples were either cleaned up using C_{18} bonded silica or were evaporated to dryness and redissolved in acetonitrile:water for analysis by HPLC. The method was validated by fortifying untreated crop samples with known amounts of fluazinam and carrying these samples through the procedure alongside the treated stored samples.

Table B.7.6.2.2-1 procedural recovery data for fluazinam in potato, coffee, onion grape and wine from fortified concurrent stability samples.

Crop	Fortification level [mg/kg]	Recoveries [%]
Potato	0.1	67, 68, 76, 84, 85, 93, 93, 102
	0.2	79, 84
Coffee	0.1	65, 72, 78, 83
	0.2	62, 66, 73, 73, 74, 82
Onion	0.1	79, 82, 95, 106
	0.2	73, 78, 80, 82, 84, 102
Grape	0.1	62, 64, 76, 81, 86, 88, 89, 94
	0.2	73, 77
Wine	50 µg/L	67, 67, 69, 72, 73, 73, 78, 82
	200 µg/L	70, 79

Based on the data shown in table B.7.6.2.2-1 it can be concluded that the analytical method applied is acceptable.

Findings:

Mean recoveries from each sampling interval are shown in table B.7.6.2.2-2.

Table B.7.6.2.2-2 Mean recoveries of fluazinam residue levels in stored crop samples fortified at 0.1 mg/kg.

Crop[1]	Storage Interval at -15 °C (days)	Storage Interval of Methanol Extract (<8 °C (days)	Mean Residue (mg/kg)[2]	Mean recovery [%]
Potato	1	10	0.12	120
	118	1	0.08	80
	218	1	0.09	90
	363	15	0.09	90
	758	14	0.08	80
Coffee	1	10	0.11	110
	107	25	0.10	100
	217	8	0.09	90
	425	37	0.10	100
	790	8	0.10	100
Onion	5	6	0.10	100
	118	16	0.08	80
	218	1	0.11	110
	481	1	0.08	80
	768	1	0.09	90
Grape	5	13	0.11	110
	113	10	0.11	110
	243	8	0.10	100
	428	8	0.09	90
	786	4	0.12	120
Wine	5	10	40	80
	111	0	48	96
	222	3	51	102
	377	1	48	96
	777	7	47	94

[1] Crop samples fortified at 0.10 mg/kg; wine fortified at 50 µg/L

[2] Average of two values

Conclusion:

No significant decrease in residue levels was found in any of the crops after storage under refrigeration at <-15 °C for up to 26 months. Based on the fortification concentration of 0.1 mg fluazinam/kg (wine: 50 µg fluazinam/L), homogenates potato, onion, coffee, grape and wine were shown to be stable through the entire storage period.

B.7.7 Effects of industrial processing and/or household preparation (Annex IIA 6.5; Annex IIIA 8.4)

No studies on the effects of industrial processing and/or household preparation have been submitted: Residues of fluazinam in potato tubers were found to be <0.01 mg/kg (LOQ) in the raw agricultural commodity. Basing on a residue definition “fluazinam – parent only”, a very low acute toxicity and an adequate high ADI, studies on the effects of industrial/household processing on nature or level of the residue are not regarded as necessary.

B.7.8 Livestock feeding studies (Annex IIA 6.4; Annex IIIA 8.3)

The results of both plant metabolism studies and residue trials demonstrate that fluazinam residues in or on potatoes are expected to be lower than 0.01 mg/kg, the limit of quantitation (LOQ) for the residue method.

The following intake calculations presuming a highest residue of 0.01 mg/kg in feed items additionally point out that the dietary burden will not exceed the trigger of 0.1 mg/kg feed (dry weight basis). In the metabolism study in lactating goat transfer factors were calculated on fresh weight basis with the highest for liver (0.0585, see table B.7.2.1-7). Even by calculating with a “default” transfer factor of 0.1 no fluazinam derived residues will be found in animal tissues >0.01 mg/kg.

Table B.7.8-1 Intake calculations* for beef cattle: Maximum daily intake of 15 kg dry matter for 350 kg bodyweight:

Actual crop	Max. percentage of actual crop in diet per day	Intake of dry matter from actual crop [kg/animal/ day]	% of dry matter in actual crop	Intake of fresh material [kg/animal/ day]	Residue in actual crop (fresh material) [mg/kg]	Residue intake [mg/animal/ day]	Residue intake [mg/kg bw/day]	Dietary burden [mg/kg diet]
Potato tubers	60	9	15	60	0.01	0.60	0.002	0.04

* according to the Document 7031/VI/95/rev.4 (22.7.1996), Appendix G, Livestock Feeding Studies

Table B.7.8-2 Intake calculations* for dairy cattle: Maximum daily intake of 20 kg dry matter for 550 kg bodyweight:

Actual crop	Max. percentage of actual crop in diet per day	Intake of dry matter from actual crop [kg/animal/ day]	% of dry matter in actual crop	Intake of fresh material [kg/animal/ day]	Residue in actual crop (fresh material) [mg/kg]	Residue intake [mg/animal/ day]	Residue intake [mg/kg bw/day]	Dietary burden [mg/kg diet]
Potato tubers	30	6	15	40	0.01	0.40	0.0007	0.02

* according to the Document 7031/VI/95/rev.4 (22.7.1996), Appendix G, Livestock Feeding Studies

In the metabolism study in lactating goat it was demonstrated that no accumulation in tissues or milk is expected. Therefore no feeding studies in ruminants are requested.

Calculation of the dietary burden for poultry basing on a highest residue of 0.01 mg/kg in potatoes does not exceed the trigger of 0.1 mg/kg feed item (dry weight basis). See table B.7.8-3.

Table B.7.8-3 Intake calculations* for poultry: Maximum daily intake of 0.12 kg dry matter for 1.9 kg bodyweight:

Actual crop	Max. percentage of actual crop in diet per day	Intake of dry matter from actual crop [kg/animal/ day]	% of dry matter in actual crop	Intake of fresh material [kg/animal/ day]	Residue in actual crop (fresh material) [mg/kg]	Residue intake [mg/animal/ day]	Residue intake [mg/kg bw/day]	Dietary burden [mg/kg diet]
Potato tubers	20	0.024	15	0.160	0.01	0.0016	0.0008	0.013

* according to the Document 7031/VI/95/rev.4 (22.7.1996), Appendix G, Livestock Feeding Studies

In the metabolism study laying hens were dosed with 0.76 mg/kg bw (10 mg/kg diet as received), which represents the 1000fold expected intake. The residue levels in eggs did not appear to reach a plateau level by the end of the study; however it was demonstrated that no accumulation in tissues or eggs is expected. Therefore no livestock feeding studies in laying hens are requested.

Conclusion of RMS:

No livestock feeding studies are required; no MRL for animal tissue is proposed.

B.7.9 Residues in succeeding crops or rotational crops (Annex IIA 6.6; Annex IIIA 8.5)

The nature and amount of uptake of fluazinam in succeeding crops was investigated in a series of three successive studies using radio-labelled fluazinam.

In the **first** report, three representative rotational crops (barley, carrots and lettuce) were grown in small plots at three plant-back intervals following fluazinam application to bare ground and

harvested. The total radioactive residue was determined, and residues were extracted and profiled in this portion of the study.
In the **second** report, the nature of the radioactive residues was examined in more detail and comparison to metabolite standards was performed.
The **third** report focused on characterization of the radioactive residue in barley grain and comparison with natural product formation, due to the extensive level of re-incorporation of radioactivity seen in the rotated crops.

B.7.9.1 Confined rotational crop study using radiolabelled material

References:
Robinson, R.A., Hoffman, S.L. (1994): Confined Rotational Crop Study on Fluazinam (IKF-1216) Part 1: Total Radioactive Residue Determination, Residue Extraction and Profiling, and Isolation and Identification of Trifluoroacetic Acid (Preliminary Report) (Report No: 5032-92-0093 XBL Report No. RPT00207).
Guidelines: EPA Pesticide Assessment Guidelines, Subdivision N Chemistry: Environmental Fate, Section 165-1.
GLP: Yes.
Study considered acceptable.

Robinson, R.A., Hoffman, S.L. (1995): Confined Rotational Crop Study on Fluazinam (IKF-1216) (Report No: 5032-92-0093 XBL Report No. RPT00207).
Guidelines: EPA Pesticide Assessment Guidelines, Subdivision N Chemistry: Environmental Fate, Section 165-1.
GLP: Yes.
Study considered acceptable.

Jentoft, N.H. (1995): Confined Rotational Crop Study on Fluazinam (IKF-1216); Re-incorporation of Radioactivity into Natural Products (Report No: 5032-92-0093 EF-002).
Guidelines: EPA Pesticide Assessment Guidelines, Subdivision N Chemistry: Environmental Fate, Section 165-1.
GLP: Yes.
Study considered acceptable.
Material and Methods:
The study was conducted to determine the nature and amount of uptake of fluazinam-derived residues in rotational crops at various time intervals in soil treated with ^{14}C-fluazinam. The ^{14}C-fluazinam was uniformly labelled in the phenyl ring or the 2,6-positions of the pyridine ring of the molecule. It was applied on a small outdoor plot with sandy loam soil. The soil was treated twice at a rate of 1.12 kg/ha per application, the total application amounted 2.24 kg/ha. For each labelled compound, three plots (0.7 m x 3.6 m) were established. Lateral confinement was achieved using a wooden frame around the areas. An identical set of control plots was constructed upwind of the treated plots. Each plot was divided into three crop sections; the

rotational crops barley, carrots and lettuce, as representatives of cereal, root and leafy crop groups, respectively were planted into the treated soil 30, 120 and 365 days after treatment (DAT). Because of crop failure, the barley planted at 30 DAT in the plot treated with phenyl-labelled fluazinam was replanted at 68 DAT.

Plant samples from the control and treated plots were harvested at the immature and mature stages.

Table B.7.9.1-1 Summary of crop harvest dates

Sample	Harvest date		
	30 DAT Planting	120 DAT Planting	365 DAT Planting
Barley			
Forage (immature)	68 DAT (pyr) 99 DAT (phe) [1]	174 DAT	420 DAT
Grain & straw (mature)	138 DAT (pyr) 174 DAT (phe) [1]	355 DAT	449 DAT
Lettuce			
immature	68 DAT	174 DAT	455 DAT
mature	89 DAT	244 DAT	477 DAT
Carrots			
immature	99 DAT	320 DAT	509 DAT
mature	155 DAT	355 DAT	534 DAT

[1] Because of crop failure, the barley in the phenyl-labelled plot was replanted at 68 DAT. This resulted in different harvest dates for the barley from the phenyl- and pyridine-labelled plots during the 30 DAT planting.

Soil core samples were taken at a depth of approximately 30.5 cm, prior to application, immediately following each application, at each rotational plantback and at harvest. Soil samples were subjected to homogenization and oxidation analysis. Levels of radioactivity were determined by combustion and LSC. The total radioactive residues in soil cores are presented in table B.7.9.1-2. No further analysis data were reported.

Crops: Crop samples for each radiolabel were individually homogenized with dry ice. Levels of radioactivity were determined by combustion and LSC.

The limit of quantitation (LOQ) for radioactive residues determined by LSC was estimated statistically using control sample results.

Sample extraction: Sub-samples of each crop homogenate were extracted three times by blending with methanol/acetone (1:1). The mixture was filtered after each blending, and the extraction solvent was combined and concentrated under vacuum until only an aqueous fraction remained. The resulting sample was partitioned three times with dichloromethane. All liquid samples were analyzed directly by LSC. The post-extraction solids (PES) were allowed to dry and were then subjected to combustion analysis. All samples generated from crop extractions

(aqueous and organic extracts as well as PES) were stored at ≤5 °C prior to HPLC analysis. Crops that were very dry (i.e. barley straw and grain) were hydrated by adding approximately 3 volumes per sample weight (v/w) of water and storing overnight at ~2 °C. The initial blending with methanol/acetone (1:1) was increased from 2 v/w to 6 v/w to allow for further hydration and sample swelling. The remainder of the extraction proceeded as described above.
Fluazinam and fifteen other compounds were used as reference chemicals for HPLC analyses.
Findings:
The residues in soil after application of 2.24 kg fluazinam/ha are summarised in table B.7.9.1-2. Looking at the 30 DAT interval, the residues in the upper soil layer found 204 days (174 + 20) after application in both radiolabels are almost as high as those found immediately after the second application. Residues in the 0-15 cm soil layer seem to decrease at a later time point which we do not know since no data are provided in this report. However, it can be seen that during the observed period only small amounts of residues were transferred into the 15-35.5 cm layer.

Table B.7.9.1-2 Residue levels of 14C-fluazinam in soil cores after application of two times 1.12 kg a.i./ha (total: 2.24 kg a.i./ha).

Days after treatment	Stage of crop/days after planting	Radioactive residue [mg-equ/kg] PYRIDYL LABEL		Radioactive residue [mg-equ/kg] PHENYL LABEL	
		Soil depth 0-15 cm	Soil depth 15-35.5 cm	Soil depth 0-15 cm	Soil depth 15-35.5 cm
Pre application	---	n.d. *	n.d.	n.d.	n.d.
Application 1 (April)	---	0.260	<LOQ **	0.117	0.008
Application 2 (May)	---	0.590	<LOQ	0.608	0.009
30	Planting	0.403	<LOQ	0.401	<LOQ
	lettuce immature / 68	0.643	<LOQ	0.372	<LOQ
	lettuce mature / 89	0.230	0.013	0.478	<LOQ
	carrot immature / 99	0.384	0.012	0.370	0.015
	carrot mature / 155	0.370	0.051	0.410	0.037
	barley forage / 68;99	0.321	<LOQ	0.378	0.018
	barley mature / 138;174	0.577	0.016	0.542	0.076
120	Planting	0.583	<LOQ	0.782	0.026
		0.309	0.052	0.305	0.024
		0.286	<LOQ	0.350	0.016
	At various crop stages sampled and shipped, but no analytical data provided.	---	---	---	---
365	At planting and at various crop stages sampled and shipped, but no analytical data provided.	No data	No data	No data	No data

* not detected

** LOQ: The limit of quantitation for soil sample oxidation analysis ranged from 91 dpm/g to 153 dpm/g, corresponding to 0.005 to 0.008 mg-equ/kg.

For lettuce and carrots, the total radioactive residue (TRR) generally decreased with increasing plantback-time. From [^{14}C-**pyridine**] fluazinam-treated soil the TRR found in lettuce, was consistently low with similar residues found at each of the plant-back intervals. Only 0.039 mg-equ/kg was observed in the 365 DAT crop. Carrot root residues decreased at each plantback interval with less than 0.010 mg-equ/kg found in the 365 DAT crop. Similar TRR levels were observed in the barley grain from the 30 DAT and 365 DAT plant-back intervals in the [^{14}C-pyridine] fluazinam-treated plots, but the levels at 120 DAT were observably lower.
In the [^{14}C-**phenyl**] fluazinam-treated plots, the residue concentrations in barley grain appeared to increase with increasing plant-back time (0.296 mg-equ/kg at 365 DAT), although subsequent analyses showed that the material was associated primarily with re-incorporation of natural products such as starch. Lettuce and carrots grown in the 365 DAT soil had only 0.040 and 0.012 mg-equ/kg, respectively. An overview on the total radioactive residues in the crops is given in table B.7.9.1-3.

Table B.7.9.1-3 Residue levels of 14C-fluazinam in rotational crops after application of two dose-rates of 1.12 kg a.i./ha (total dose: 2.24 kg a.i./ha).

Sample	30 DAT Planting		120 DAT Planting		365 DAT Planting	
	Phenyl label [mg-equ/kg]	Pyridyl label [mg-equ/kg]	Phenyl label [mg-equ/kg]	Pyridyl label [mg-equ/kg]	Phenyl label [mg-equ/kg]	Pyridyl label [mg-equ/kg]
Lettuce immature	0.318	0.119	0.470	0.036	0.104	0.049
Lettuce mature	0.282	0.065	0.174	0.034	0.040	0.039
Barley forage	0.135	0.327	0.934	0.075	0.529	0.138
Barley grain	0.054	0.234	0.155	0.065	0.296	0.228
Barley straw	0.093	1.249	0.256	0.105	0.273	0.266
Carrot roots immature	0.101	0.087	0.066	0.036	0.015	0.010
Carrot tops immature	0.429	0.333	0.164	0.045	0.056	0.059
Carrot roots mature	0.070	0.045	0.066	0.024	0.012	<0.010
Carrot tops mature	0.349	0.222	0.223	0.034	0.040	0.057

Note: values are listed from the extraction-distribution-summaries, as there are only LSC data for the 30 DAT planting. These LSC data correspond with the values shown in Table B.7.9.1-2

All of the plant samples with TRR values above 0.010 mg/kg were subjected to extraction. The organosoluble residues in the edible portions of crops used for human consumption (lettuce, carrot roots, barley grain) were very low and generally below 0.01 mg/kg in mature crops (with the exception of the 365 DAT barley grain). Organosoluble residues from crop fractions used as animal feed items were more variable. The highest residues detected were in pyridine-labelled barley straw (0.081 mg/kg). However, the phenyl-labelled barley straw from the same sampling time had organosoluble residues below 0.01 mg/kg, indicating that the residues present in the

pyridine-labelled fraction were extensively degraded and no longer contained the fluazinam structural backbone.

The results from the mature crop samples are shown in Table B.7.9.1-4:

Table B.7.9.1-4 Partitioning of Fluazinam in Rotational Crop Fractions

Fraction	Plant-back Interval					
	30 DAT		120 DAT		365 DAT	
	%	mg/kg	%	mg/kg	%	mg/kg
^{14}C-Phenyl label						
Mature Lettuce						
Organic	0.00	<0.001	1.69	0.003	5.58	0.002
Aqueous	94.81	0.267	93.50	0.163	62.41	0.025
PES	5.19	0.015	4.81	0.008	32.01	0.013
Mature Carrot Roots						
Organic	9.55	0.007	8.93	0.006	28.03	0.003
Aqueous	82.37	0.057	78.29	0.052	48.21	0.006
PES	8.08	0.006	12.78	0.008	28.76	0.003
Mature Carrot Tops						
Organic	2.63	0.009	2.41	0.005	10.58	0.004
Aqueous	85.83	0.300	89.16	0.199	40.63	0.016
PES	11.54	0.040	8.43	0.019	48.79	0.020
Barley Grain						
Organic	8.14	0.004	2.41	0.004	3.73	0.011
Aqueous	40.98	0.023	75.40	0.117	58.96	0.175
PES	50.88	0.027	22.19	0.034	37.31	0.110
Barley Forage						
Organic	25.78	**0.035**	2.23	0.021	6.96	0.037
Aqueous	53.74	0.072	94.26	0.880	77.30	0.409
PES	20.48	0.028	3.51	0.033	15.74	0.083
Barley Straw						
Organic	8.06	0.007	2.65	0.007	6.00	**0.016**
Aqueous	57.84	0.054	82.58	0.211	59.54	0.163
PES	34.10	0.032	14.77	0.038	34.46	0.094
^{14}C-Pyridyl label						
Mature Lettuce						
Organic	8.74	0.006	9.74	0.003	8.41	0.003
Aqueous	48.71	0.031	42.74	0.014	42.66	0.017
PES	42.55	0.028	37.83	0.013	48.93	0.019
Mature Carrot Roots					Not extracted (TRR	

Fraction	Plant-back Interval					
	30 DAT		120 DAT		365 DAT	
	%	mg/kg	%	mg/kg	%	mg/kg
Organic	14.11	0.006	14.06	0.003	<0.010 mg/kg)	
Aqueous	54.86	0.025	60.31	0.015		
PES	31.03	0.014	25.63	0.006		
Mature Carrot Tops						
Organic	10.94	0.024	10.37	0.004	14.41	0.008
Aqueous	34.72	0.077	47.00	0.016	34.33	0.020
PES	54.34	0.121	42.63	0.014	51.26	0.029
Barley Grain						
Organic	3.70	0.009	4.43	0.003	5.00	0.011
Aqueous	5.10	0.012	27.02	0.017	19.83	0.046
PES	91.20	0.213	68.55	0.045	75.17	0.171
Barley Forage						
Organic	11.48	**0.037**	15.32	0.011	6.39	0.009
Aqueous	62.32	0.204	61.95	0.046	61.55	0.085
PES	26.20	0.086	19.43	0.015	29.85	0.041
Barley Straw						
Organic	6.50	0.081	6.67	0.007	6.00	**0.016**
Aqueous	50.31	0.629	34.69	0.037	51.34	0.137
PES	43.19	0.539	45.22	0.047	42.66	0.113

More than 90% of the TRR in the mature **lettuce** grown in **phenyl**-labelled fluazinam treated soil at the 30 DAT and 120 DAT plant-back intervals was aqueous extractable. Little or no residues were found in the organic extract or the PES (post extraction solids). Lettuce grown at the 365 DAT plant-back interval had less extractable residues than the 30 DAT and 120 DAT crops. About 40-50% (max. 0.028 mg-equ/kg) of the residues in lettuce grown in **pyridyl**-labelled fluazinam treated soil was found in the PES at all plant-back intervals. Organo-extractable residues accounted for about 10% of the TRR while the aqueous fraction accounted 40-50% (max. 0.031 mg-equ/kg) of the TRR.

In **carrots** grown in **phenyl**-labelled fluazinam treated soil, approximately 80% of the TRR was aqueous extractable from carrot roots at the 30 DAT and 120 DAT plant-back intervals. About 10% of the residues were found in both the PES and the organic extract. Extractable residues and PES from carrots grown in the 365 DAT soil were below 0.010 mg-equ/kg. In carrots grown in **pyridyl**-labelled fluazinam treated soil 50-60% of the TRR was found in the aqueous extract, but the highest concentration was only 0.025 mg-equ/mg in the 30 DAT crop. Less than 0.006 mg-

equ/kg were found in the organic extracts. The 365 DAT carrot root sample was not analysed because the TRR level was below 0.010 mg-equ/kg. In carrots, generally less residues were found in the PES as compared to lettuce.

In **barley grain** grown in **phenyl**-labelled fluazinam treated soil, approximately 20-50% of the TRR remained bound in the PES after extraction. The amount of bound residue was generally higher than that found in lettuce or carrots. About 40-75% of the residue was aqueous extractable with less than 10% found in the organic extract at all plant-back intervals. Extractable residues from barley grain grown in **pyridyl**-labelled fluazinam treated soil accounted for less than 32% of the TRR at any plant-back interval as compared to the extractable residues found in lettuce and carrots. In fact, less than 10% of the 0.234 mg-equ/kg TRR level from the 30 DAT plant-back interval was extractable. Most of the extractable residues were found in the aqueous fraction with a maximum of 5% partitioned into the organic layer.

Analysis of the organic extracts:

The organosoluble residues in the edible portions of crops used for human consumption (namely lettuce, carrot roots, barley grain) were very low and generally below 0.01 mg/kg in mature crops (with the exception of the 365 DAT barley grain with 0.011 mg-equ/kg in both labels). Organosoluble residues from crop fractions used as animal feed items were more variable. Except for the organic fraction of DAT 365 pyridine labelled barley forage (<0.01 mg-equ/kg), residue levels detected in barley forage were 0.011 to 0.037 mg-equ/kg for both labels. The highest organosoluble residues detected were from the pyridine-labelled barley straw from DAT 30 with 0.081 mg-equ/kg. However, the phenyl-labelled barley straw from the same sampling time had organosoluble residues below 0.01 mg/kg, indicating that the residues present in the pyridine-labelled fraction were extensively degraded and no longer contained the fluazinam structural backbone.

HPLC profiles of the organic fractions showed radioactive regions of radioactivity eluting between 15 and 25 minutes, which did not contain any single major peak but rather indicated the presence of many compounds. Some metabolites containing the intact ring system may have been present. Based on HPLC retention time, HYPA and CAPA were the only possible reference compounds that could be present in these samples. As fluazinam related metabolites (i.e. those with intact ring system) should be present in samples of both labels at approximately equal levels, only the CH_2Cl_2 fractions from DAT 30 barley forage (0.035 mg-equ/kg phenyl label and 0.037 mg-equ/kg pyridine label) and from DAT 365 barley straw (both labels 0.016 mg-equ/kg) could contain a significant amount of fluazinam related metabolites. HPLC analysis of these fractions did not detect any major peaks <0.01 mg-equ/kg that were consistent with any of the potential fluazinam related reference compounds. Further identification and characterisation was not possible due to too low quantities of organosoluble metabolites, especially at the later planting intervals.

Analysis of the aqueous extracts:

HPLC profiles of the aqueous extracts of crops grown in **phenyl-labelled** fluazinam treated soil showed **in general one major radioactive peak.** This single peak accounted for 60 **to 100% of**

the aqueous extract. The profiles of the aqueous extracts of crops grown in pyridine-labelled fluazinam treated soil in contrast showed two areas of radioactivity with significantly more radioactivity in the latter region. None of the observed metabolites co-chromatographed with the possible metabolites that contain both ring systems, indicating that fragmentation between the two radio-labelled aromatic and hetero-aromatic rings occur. Additionally, concerning the reference compounds that contained only the pyridine ring, the only possible matches (3,2,5-CHTF and 2,5-HTF) had lost the amino group and possibly the chlorine atom.
The phenyl and pyridine moieties of fluazinam were separated and extensively metabolised.

Analysis of the major metabolite in crops grown in phenyl-labelled fluazinam treated soil:

The aqueous fraction from the DAT 174 barley forage (of the DAT 120 planting) was selected to be further analysed due to the magnitude of TRR (0.880 mg-equ/kg), the large amount of sample available and because its HPLC profile was consistent with the profiles derived from barley grain, lettuce and carrot roots. This aqueous sample was subjected to several chromatographic purification methods; the results of these procedure indicated that the radioactive residues appeared to be only one compound. This purified fraction was acidified and the ^{14}C residues were extracted into ether. **GC/MS** analysis confirmed the presence of [^{14}C]-TFAA in this ether fraction. Co-chromatography by **HPLC** of the same fraction with unlabelled TFAA also identified this single metabolite as **TFAA**. As the HPLC profile of the sample was consistent with the profiles derived from barley grain, lettuce and carrot roots, the entire radioactive residue of the aqueous fractions was consequently understood to be TFAA.

Table B.7.9.1-5 Amounts of TRR of the aqueous fractions of edible crops grown in [14C-phenyl]fluazinam treated soil representing TFAA(trifluoroacetic acid):

Crop	Planting interval					
	DAT 30		DAT 120		DAT 365	
	[%]	[mg-equ/kg]	[%]	[mg-equ/kg]	[%]	[mg-equ/kg]
Lettuce	94.81	0.267	93.50	0.163	52.80	0.021
Carrot roots	82.37	0.057	73.39	0.049	35.48	0.004
Barley grain	37.48	0.021	74.21	0.115	58.96	0.175

Analysis of the major metabolite in crops grown in pyridine-labelled fluazinam treated soil:

As noted above, compounds containing both intact ring systems have been eliminated as possible metabolites by HPLC profiling. Additionally, of the reference compounds that contained only the intact pyridine ring, only 3,2,5-CHTF and 2,5-HTF are possibilities for metabolites that elute in the 15-20 minute region. Additional work was done to further identify and characterize these metabolites. The aqueous fraction from the DAT 138 barley straw (of the DAT 30 planting) was selected to be further analysed due to the magnitude of TRR (0.629 mg-equ/kg) and the large amount of sample available for extraction. Characterization of the fraction by fluorine NMR analysis indicated that the molecule did not have a trifluoromethyl substituent attached to the

pyridine ring. To determine if these metabolites represented compounds with the intact pyridine ring, the fraction was further purified by using solid phase extraction and in the following the resulting fractions subjected to derivatisation and spectroscopic analyses. These analyses demonstrated that there were at least two components resulting from ring opening and fragmentation.

Analysis of non-extractable residues:

The PES (post extraction solids) from the mature crops and barley forage of the DAT 365 plantings were subjected to extensive series of procedures in an attempt to release the residues. Cellulase hydrolysis succeeded in releasing up to 51% of the PES. Analyses of the aqueous fractions from enzyme hydrolysis indicated two regions of radioactivity by HPLC at about 5 and 15 minutes. Subsequent mild acid , strong acid and strong base reactions succeeded in releasing most of the non-extractable residues. After base hydrolysis the resulting PES-fractions were all <10% of the original radioactive residue and <0.01 mg-equ/kg: The only exception was the phenyl-labelled barley straw which had a value of 0.011 mg-equ/kg.

A sample of **pyridine-labelled barley grain** from the DAT 30 planting was extracted with methanol/acetone (1:1). The PES fraction was then treated with hot water to gelatinize the starch. This mixture was cooled and incubated twice with α-amylase. This enzyme treatment solubilized approximately 43% of the initial amount of the PES. HPLC analysis of the supernatant solution indicated that about 38% of the original weight of the PES was recovered as maltose and glucose. This mixture of sugars was hydrolysed to convert maltose and other oligosaccharides to glucose. The glucose was then reduced to sorbitol and acetylated to give sorbitol hexa-acetate. Each step of the reaction was monitored by HPLC to ensure that the expected products were obtained. Further LSC analyses steps showed that approximately 15% of the starting radioactivity was recovered as sorbitol hexa-acetate with no correction for losses due to sampling or incomplete reaction. Correcting for these losses indicates that at least 32% of the initial radioactivity was present as glucose in starch.

It can be assumed that the provided data demonstrate that some portion of the radioactivity from ^{14}C pyridine labelled fluazinam is incorporated into the glucose moieties of starch. Glucose is the precursor for biosynthetic reactions in plants. If glucose is labelled, other naturally occurring compounds synthesized by the plant could also be labelled as they are finally derived from glucose.

Storage Stability:

To establish that crop samples were stable in storage at ≤10 °C for prolonged periods, an immature lettuce sample from the 30 DAT planting was re-extracted after more than two years of storage using identical extraction conditions to the original. The distribution of radioactivity was very close to the original extraction. Comparison of the HPLC chromatograms of the aqueous fractions from the re-extraction with those originally obtained was also very similar. In addition, re-analysis of the original aqueous fractions after two years of storage at ≤5 °C showed close similarity to the original. Since these storage intervals were longer than any periods encountered in the course of the laboratory phase of the study, it was demonstrated that there were no

problems associated with storage over the course of the study.

Conclusion:

Soil:

Based on the data submitted in this study it cannot be excluded that fluazinam or related residues are persistent in soil (see Table B.7.9.1-2: Residue levels of ^{14}C-fluazinam in soil cores after application of two times 1.12 kg a.i./ha (total: 2.24 kg a.i./ha).

Transition factors:

To describe the potential uptake of radioactive residues by plants from the soil the following transition factors were calculated for the 30 DAT plant-back interval:

Table B.7.9.1-6 Calculated uptake of 14C-fluazinam residues from soil during 30 DAT plant-back interval (transition factors)

Residues in soil (0-15 cm) [mg-equ/kg]	crop	Plant residues [mg-equ/kg]	Transition factor (plant/soil)	
			at planting	at harvest
Pyridyl label				
0.403 (day 30, planting) 0.230 (day 89, harvest)	Lettuce	0.065	0.16	0.28
0.403 (day 30, planting) 0.370 (day 155, harvest)	Carrot root	0.045	0.11	0.12
0.403 (day 30, planting) 0.577 (day 174, harvest)	Barley grain Barley straw	0.234 1.249	0.58 3.10	0.41 2.16
Phenyl label				
0.401 (day 30, planting) 0.178 (day 89, harvest)	Lettuce	0.282	0.70	1.58
0.401 (day 30, planting) 0.410 (day 155, harvest)	Carrot root	0.070	0.17	0.17
0.401 (day 30, planting) 0.542 (day 174, harvest)	Barley grain Barley straw	0.054 0.093	0.13 0.23	0.01 0.02

The uptake ratios from soil to plant <1.0 (related to the radioactivity in soil at the time of planting) show that noticeable but little uptake of radioactive residues via the soil occurs. In the pyridyl

labelled plot uptake ratios >1.0 are found for barley straw (related to both planting and harvest). Barley straw also showed significant radioactive residues, which can be explained by the weight reduction due to the loss of water (increasing artificially the radioactive concentration in the plant part). In the phenyl labelled plot the uptake ratio of >1.5 for lettuce related to harvest describes clearly that uptake of radioactive residues from soil to lettuce occurs, while the radioactive concentration in soil is decreasing.

As the highest uptake ratio (transition factor) for edible crop parts is 1.58 and the highest uptake ratio for dried plant parts is 3.1, it can be considered that fluazinam was not accumulated after uptake from soil.

Residues in rotational crops:

Residues in rotational crops represent fragments from either the phenyl or pyridine ring structure resulting from extensive metabolic degradation. Organo-extractable residues were low in barley forage and straw; no fluazinam-related compounds (metabolites still retaining the basic fluazinam structural two-ring moiety) ≥0.01 mg/kg were detected. The low levels of radioactivity in most crop fractions, especially at the later planting intervals, precluded extensive investigation. Parent fluazinam was not detected in any extract from any crop sample.

Analysis of the non-extractable residues showed that after base hydrolysis the resulting PES-fractions were all <10% of the original radioactive residue and <0.01 mg/kg: The only exception was the phenyl-labelled barley straw which had a value of 0.011 mg/kg. Characterization of the non-extractable residue from the barley grain (DAT 30) demonstrated that ^{14}C from fragmentation of the pyridine ring had been re-incorporated into natural products such as starch.

However, in the **aqueous fractions** significant radioactive residues in edible parts of plants were found. These residues were identified as trifluoroacetic acid **TFAA** amounting **>0.1 mg/kg** in lettuce (0.27 mg/kg at DAT 30; 0.16 mg/kg at DAT 120) and in barley grain (0.12 mg/kg at DAT 120; 0.18 mg/kg at DAT 365; amount increasing with planting interval).

B.7.9.2 Crop rotation studies (field test)

No field studies in succeeding crops have been submitted: Metabolism studies on rotational crops have shown that fluazinam or fluazinam related metabolites (containing the intact ring system) are not accumulated by plants after uptake from soil. Provided a provisional residue definition “fluazinam – parent only” residues remained below any relevant level. The substance is not translocated into deeper soil layers. Since the test substance was applied to bare soil at the maximum field rate, residues in plants could be even lower under conditions of normal agricultural practice. As fluazinam and its metabolites will represent only little risk of uptake by succeeding crops or leaching into deeper soil layers, field studies in succeeding crops are not regarded to be necessary for the moment.

B.7.9.3 Overall conclusion

The possible intake of soil derived fluazinam residues via succeeding crops was investigated by 3

studies using radiolabelled fluazinam, the test substance was applied to bare soil at the maximum field rate. Total radioactive residues declined with the last planting interval, fluazinam was not accumulated by plants after uptake from soil. Residues of fluazinam or related residues (still retaining the basic fluazinam structural two-ring moiety) in edible parts of plants remained below any relevant level. The main part of the radioactivity recovered was found to be trifluoro-acetic acid (TFAA) at a significant level up to 0.27 mg/kg. As no information on the toxicological significance of TFAA is available, no final conclusion can be drawn if this component has to be included in a residue definition for rotational crops. Provided an inclusion of TFAA in the residue definition for rotational crops, either supervised field trials in succeeding crops should be required or restrictions with regard to appropriate plant-back intervals for rotational crops should be considered.

However, the active substance was not translocated into deeper soil layers but it could be observed that fluazinam or related residues were persistent in the upper soil layers. Not regarding the residues of TFAA, it is concluded that the application of fluazinam according to the intended application rates will not lead to detectable residues.

B.7.10 Proposed pre-harvest intervals for envisaged uses, or withholding periods, in the case of post harvest uses (Annex IIA 6.8; Annex IIIA 8.7)

B.7.10.1 Pre-harvest intervals for each relevant crop

Based on the information on the intended use provided by the notifier, the following pre harvest interval can be set:

Table B.7.10.1-1: PHI proposed by the notifier after application of fluazinam:

Crop	Food/feed item	PHI [days]
potatoes	tubers	7 days

B.7.10.2 Re-entry period for livestock to areas to be grazed

Not appropriate because the treated crops are not used for grazing.

B.7.10.3 Re-entry period for man to crops, buildings or spaces treated

Based on the results of the corresponding toxicological studies, it is safe for workers to return to the treated area immediately (one hour) after treatment.

B.7.10.4 Withholding period for animal feeding stuffs

Potatoes are part of animal diet. However, due to the zero residue situation in potato tubers (which are the possible feed items), it is not necessary to set a withholding period for animal feeding stuffs.

B.7.10.5 Waiting period between application and handling treated product

Based on the results of the corresponding toxicological studies, the conditions of usage of fluazinam as proposed, including a pre-harvest interval of 7 days, pose no risk for handling treated potatoes.

B.7.11 Community MRL and MRLs in EU Member States (Annex IIIA 12.2)

No harmonised EU-MRLs are established at the moment.

B.7.12 Proposed EU MRLs and justification for the acceptability of those MRLs (Annex IIA 6.7; Annex IIIA 8.6)

B.7.12.1 Potatoes

The results of 13 trials (Northern Region) and 3 trials (Southern Region) are regarded as relevant according to the supported uses. Since at harvest no residues above the LOQ could be detected in any of the potato tuber samples analysed, a **MRL of 0.01 mg/kg** (LOQ) expressed as parent compound is proposed for potatoes.

B.7.12.2 Food of animal origin

Based on the intended use, the residue data provided (according to the provisional residue definition) and the fact that no accumulation of residues from animal feed was observed in livestock metabolism studies, no MRL for food of animal origin is proposed.

B.7.13 Proposed EU import tolerances and justification for the acceptability of those residues

No import tolerances are established.

B.7.14 Basis for differences, if any, in conclusions reached having regard to established or proposed CAC MRLs

There are no CAC MRLs established.

B.7.15 Estimates of potential and actual dietary exposure through diet and other means (Annex IIA 6.9; Annex IIIA 8.8)

No fluazinam residues above 0.1 µg/L are expected to occur in drinking water. Fluazinam residue intake through drinking water can therefore be assessed as theoretical maximum:
0.2 µg fluazinam/person/day assuming a daily consumption of 2 L water. This contribution is considered marginal compared to the theoretical maximal intake of fluazinam through food of plant and animal origin.

B.7.15.1 Estimation of the potential and actual exposure through diet

B.7.15.1.1 Estimation of chronic exposure through diet

The theoretical maximum daily intake (TMDI) of fluazinam was calculated based on the MRL derived from residues found in those trials which were regarded as relevant. A maximum residue level (MRL) for potatoes is proposed at 0.01 mg/kg. The proposed **ADI-value**, based on the results of a 52 week study in dogs, was set to **0.01 mg/kg bw/d**.

The diet consumption data were taken from

a) the European diet compiled in the WHO Publication on Cultural and Global Diets, 1994 (for an adult of 60 kg bodyweight) and from

b) the German diet given in the BBA-Guideline Part IV, 3-7, 1993 (for a 4-6 year old girl of 13.5 kg bodyweight).

a) TMDI-calculation (diet consumption data obtained from the European diet compiled in the WHO Publication on Cultural and Global Diets, 1994

Table B.7.15.1.1-1: Theoretical Maximum Daily Intake (TMDI) of fluazinam residues

Food	Average daily consumption of food item [g/day]	MRL[1)] [mg/kg]	TMDI [mg/person/day]
Potatoes	240,8	0.01	0.002408
TMDI [mg/person/day]			0.002408
TMDI [mg/kg bw/day]			0.000040

1) Residues expressed as mg fluazinam /kg

TMDI: 0.000040 mg/kg bw/day

Percent of ADI: 0,4 %

b) TMDI-calculation (diet consumption data obtained from the German diet given in the BBA Guideline, Part IV, 3 – 7. 1993)

The results of the TMDI-estimation of fluazinam residues through food of plant and animal origin are summarised in table B.7.15.1.1-2:

Table B.7.15.1.1-2: Theoretical Maximum Daily Intake (TMDI) of fluazinam residues

Food	Average daily consumption of food item [g/day]	MRL[1)] [mg/kg]	TMDI [mg/person/day]
Potatoes	71.1	0.01	0.000711

Food	Average daily consumption of food item [g/day]	MRL[1)] [mg/kg]	TMDI [mg/person/day]
TMDI [mg/person/day]			0.000711
TMDI [mg/kg bw/day]			0.000053

1) Residues expressed as mg fluazinam /kg

TMDI: **0.000053 mg/kg bw/day**

Percent of ADI: **0.5 %**

No NEDI calculations are considered necessary since the ADI-contribution based on the TMDI is very low.

B.7.15.1.2 Estimation of acute exposure through diet

The results of the acute dietary risk assessment are summarised in table B.7.15.2-1. The Acute Reference Dose is proposed as 0.02 mg/kg bw, basing on the results of a rabbit developmental study. The acute intake for potatoes does not exceed the ARfD.

Table 7.15.1.2-1 Acute risk assessment

Commodity	Adult		Toddler	
	Acute intake (IESTI) [mg/kg bw/day] [1)]	% ARfD	Acute intake (IESTI) [mg/kg bw/day] [1)]	% ARfD
Potatoes	0.000259	1.3	0.0010503	5.3

1) Residues expressed as mg fluazinam /kg

B.7.15.2 Estimation of the potential and actual exposure through other means

No fluazinam residues above 0.1 µg/L are expected to occure in drinking water. Fluazinam residue intake through drinking water can therefore be assessed as theoretical maximum: 0.2 µg fluazinam/person/day assuming a daily consumption of 2 L water. This contribution is considered marginal compared to the theoretical maximal intake of fluazinam through food of plant and animal origin.

B.7.16 Summary and evaluation of residue behaviour (Annex IIA 6.10; Annex IIIA 8.9)

Storage stability:

No significant decrease in residue levels was found in potatoes after storage under refrigeration at <-15 °C for up to 26 months.

Metabolism of fluazinam in plants:

Extent and nature of residues in plant material after application of fluazinam were tested in potatoes at the normal maximum application rate of 2.02 kg phenyl labelled a.i./ha and 1.72 kg

pyridyl labelled a.i./ha.

Overall levels of ^{14}C residue in potato tubers were extremely low. The highest residue levels were found in the pyridyl level treated potatoes (0.025 mg equ/kg).

Investigations on the nature of the residue showed that the parent compound accounted for 2.3 and 5.9% of the TRR; TFAA (trifluoro-acetic acid) accounted for 0.9 and 0.6% of the TRR.

The fact that radioactivity from both labelled fluazinam appeared in starch indicated that both rings were broken down into fragments that could enter the carbon pool.

Additionally provided metabolism studies for fluazinam in peanuts, grapes and in apples also resulted in extremely low overall levels of ^{14}C residue and demonstrated that the end products of metabolism involved re-incorporation of ^{14}C from fluazinam into natural products, including starch and fatty acids.

Metabolism in livestock:

Following oral administration of ^{14}C- Fluazinam (IKF-1216) to two lactating goats at a nominal dose level of 0.33 mg/kg body weight per day (phenyl label) and 0.36 mg/kg body weight per day (pyridyl label) once daily for four consecutive days, concentrations of radioactivity in milk were low, reaching a maximum of 0.071 mg equivalents/kg following the fourth dose. A plateau level in milk was reached within the run of the study. Concentrations of radioactivity in kidney and liver were 0.047 and 0.661 mg equivalents/kg, respectively. Levels of radioactivity in muscle and fat were 0.030 and 0.211 mg equivalents/kg respectively.

Although in this study the concentration in feed (average of both labels: 11.3 mg/kg feed as received) represented more than 30 times the calculated dietary burden basing on the highest residue found in potatoes, only little transfer into edible tissues and milk was observed; residues in meat and milk after uptake from fluazinam in animal feed will not exceed the LOQ if the active substance is used according to the supported GAP.

The nature of the residue studies in lactating goats show that the major residues were the reduction products AMPA and DAPA and their sulfamate conjugates. These residues were similar to residues identified after oral administration of fluazinam to rats (the AMPA and DAPA conjugates identified from rats were glucuronides). Parent fluazinam was not detected in any of the samples.

Following oral administration of ^{14}C- Fluazinam (IKF-1216) to two groups of laying hens at a nominal dose level of 0.76 mg/kg body weight per day (phenyl and pyridyl label each) once daily for four consecutive days, concentrations of radioactivity in eggs reached a maximum of 0.388 mg equivalents/kg following the fourth dose. A plateau level in eggs was not reached within the run of the study. Concentration of radioactivity in liver was 0.984 mg equivalents/kg. Levels of radioactivity in dark muscle and fat were 0.053 and 0.948 mg equivalents/kg respectively.

Although in this study the concentration in feed (average of both labels: 10.35 mg/kg feed as received) represented more than 800 times the calculated dietary burden basing on the highest residue found in potatoes, only little transfer into edible tissues and eggs was observed; residues in meat and eggs after uptake from fluazinam in animal feed will not exceed the LOQ if the active substance is used according to the supported GAP. In laying hens, the major metabolites were

reduction products, primarily AMPA, DAPA, MAPA and HYPA, as well as measurable amounts of fluazinam parent molecule. Other metabolites found also included a mixture of conjugates representing the products of glutathione conjugation and subsequent metabolism.

Supervised field trials:

No detectable residue levels of fluazinam at or above the LOQ of 0.01 mg/kg have been determined in the potato tubers at harvest in all of the field trials. This is consistent with the results obtained in the potato plant metabolism study.

Rotational crops:

No accumulation of fluazinam related residues (metabolites still retaining the basic fluazinam structural two-ring moiety) in rotational crops after uptake from treated soil was observed. Residues represented fragments from either the phenyl or pyridine ring structure resulting from extensive metabolic degradation. In organo-extractable residues no fluazinam-related compounds ≥0.01 mg/kg were detected. Characterization of the non-extractable residue demonstrated that ^{14}C from fragmentation of the pyridine ring had been re-incorporated into natural products such as starch.

In the aqueous fractions significant radioactive residues in edible parts of plants were found. These residues were identified as trifluoroacetic acid TFAA amounting >0.1 mg-equ/kg in lettuce (0.27 mg-equ/kg at DAT 30; 0.16 mg-equ/kg at DAT 120) and in barley grain (0.12 mg-equ/kg at DAT 120; 0.18 mg-equ/kg at DAT 365; amount increasing with planting interval).

TFAA was not found in soil metabolism and it was not determined neither determined in livestock nor in laboratory animal metabolism.

Based on the no residue situation in potato tubers and on the proposed application scenarios, a MRL of 0.01 mg/kg was established for potato tubers.

No MRL was established for animal tissue.

On the basis of this MRL value, the TMDI calculation results in an exhaustion of the ADI (0.01 mg/kg bw/day) of 0.4 to 0.5%; the IESTI calculation results in an exhaustion of the ARfD (0.02 mg/kg bw) of 1.3 to 5.3%.

The consumer risk due to uptake of fluazinam residues can therefore be regarded as negligible.

Table B.7.16-1: Key results of crop residue trials conducted with fluazinam (with relevance to MRL-estimation)

Crop	Application [kg a.i./ha]	Region[1)]	No. of trials	PHI [days]	Lowest residue at harvest [mg/kg][2)]	Highest residue at harvest [mg/kg][2)]
potatoes	11 x 0.200	N	13	0-7-14	<0.01	<0.01
potatoes	6 x 250	S	3	14-15	<0.01	<0.01

1) N: Northern Region of Europe; S: Southern Region of Europe
2) Residues expressed as fluazinam

Table B.7.16-2: MRL proposals for fluazinam (based on the intended uses)

Commodity	Proposed EU-MRLs according to the intended uses [mg/kg][1)]	Comments
Potato (tubers)	0.01 *	---

1) Residues expressed in mg fluazinam /kg

B.7.17 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
Annex II Data and Information					
AII, 6a/01	McFall, D. D.	1999	Stability of Fluazinam in Potatoes and Potato Processed Fractions after Freezer Storage – Final Report. Ricerca, Inc., Report No. 6166-94-0148-CR-002 GLP/GEP Yes unpublished	Y	ISK
AII, 6a/02	Ryan, J., Sapiets, A.	1993	Fluazinam: Storage Stability of the Residues in Frozen Crop Samples. – Final Report. Zeneca Agrochemicals, Report No. RJ1538B GLP/GEP Yes unpublished	N	ISK
AII, 6b/01	Ryan, J., Sapiets, A.	1993	Fluazinam: Storage Stability of the Residues in Frozen Crop Samples. – Final Report. Zeneca Agrochemicals, Report No. RJ1538B GLP/GEP Yes unpublished	N	ISK
AII, 6.1/01	Jentoft, N. H.	1997	[^{14}C]IKF-1216 (Fluazinam) Plant Metabolism Study in Potatoes. Ricerca, Inc., Report No. 6775-96-0053-EF-001 GLP/GEP Yes unpublished	N	ISK
AII, 6.1/02	Hartman, D. A.	1995	A Peanut Plant Metabolism Study with [^{14}C]IKF-1216 (Fluazinam); (Final Report). Ricerca, Inc., Report No. 5012-91-0330-EF-002 GLP/GEP Yes unpublished	N	ISK
AII, 6.1/03	Flückiger, J.	1993	^{14}C-Fluazinam: Plant Metabolism Study in Field Grown Grape Study A (1992) [Biological Phase]. RCC Umweltchemie AG, Report No. 328735 GLP/GEP Yes unpublished	N	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
All, 6.1/04	Neal, T. R.	1996	[^{14}C]Fluazinam: Plant Metabolism Study in Field Grown Grape - Study A (1992) [Analytical Phase] Final Report. Ricerca, Inc., Report No. 5431-92-0423-EF-003 GLP/GEP Yes unpublished	N	ISK
All, 6.1/05	McClanahan, R.H.	1996	^{14}C-IKF-1216 (Fluazinam): Plant Metabolism Study in Apple Trees. Ricerca, Inc., Report No. 6021-94-0050-EF-001 GLP/GEP Yes unpublished	N	ISK
All, 6.2/01	Cheng, T.	1993/ 1994	Nature of the Residue of ^{14}C-Fluazinam (IKF-1216) in Lactating Goats (Part 1: Animal Dosing, Sample Collection and Radiochemical Analysis, Part 2: Metabolite Identification and Characterization. [redacted] Report No. 5248-92-0116-EF GLP/GEP Yes unpublished	N	ISK
All, 6.2/02	Cheng, T.	1995	Nature of the Residue of ^{14}C-Fluazinam (IKF-1216) in Laying Hens. [redacted] Report No. 5309-92-0241-EF GLP/GEP Yes unpublished	N	ISK
All, 6.3/01	Ryan, J., Sapiets, A.	1991	Fluazinam: Residue Levels in Potatoes from Trials Carried Out in Germany During 1990. ICI Agrochemicals, UK, Report No. RJ1023B GLP/GEP Yes unpublished	N	ISK
All, 6.3/02	Ryan, J., Sapiets, A.	1991	Fluazinam: Residue Levels in Potatoes from Trials Carried Out in Germany During 1989. ICI Agrochemicals, UK, Report No. RJ1025B GLP/GEP Yes unpublished	N	ISK
All, 6.3/03	Clarke, D.M.	1997	Fluazinam: Residue Levels in Early Potatoes from Trials Carried Out in the United Kingdom During 1996. Zeneca Agrochemicals, UK, Report No. RJ2308B GLP/GEP Yes unpublished	N	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
AII, 6.3/04	Burke, S.R., Sapiets, A.	1992	Fluazinam: Residue Levels in Potatoes from Trials Carried Out in the United Kingdom During 1989. ICI Agrochemicals, UK, Report No. RJ1161B GLP/GEP Yes unpublished	N	ISK
AII, 6.3/05	Dvorak, R.S., Kenyon, R.G.	1996	Magnitude of Residue of Fluazinam in Potatoes – Greece – 1995. Ricerca, Inc. USA, Report No. 6530-96-0020-CR-001 GLP/GEP Yes unpublished	N	ISK
AII, 6.3/06	Kenyon, R.G.	1998	Magnitude of Residue of Fluazinam in Potatoes – Greece – 1996. Ricerca, Inc. USA, Report No. 6953-97-0087-CR-001 GLP/GEP Yes unpublished	N	ISK
AII, 6.6/01	Robinson, R.A., Hoffman, S.L.	1994	Confined Rotational Crop Study on Fluazinam (IKF-1216) Part 1: Total Radioactive Residue Determination, Residue Extraction and Profiling, and Isolation and Identification of Trifluroacetic Acid (Preliminary Report). XenoBiotic Laboratories, Inc., Report No. 5032-92-0093 XBL Report No. RPT00207 GLP/GEP Yes unpublished	N	ISK
AII, 6.6/02	Robinson, R.A., Hoffman, S.L.	1995	Confined Rotational Crop Study on Fluazinam (IKF-1216). XenoBiotic Laboratories, Inc., Report No. 5032-92-0093 XBL Report No. RPT00207 GLP/GEP Yes unpublished	N	ISK
AII, 6.6/03	Jentoft, N.H.	1995	Confined Rotational Crop Study on [^{14}C]Fluazinam (IKF-1216) Re-incorporation of Radioactivity into Natural Products. Ricerca, Inc., Report No.5032-92-0093-EF-002 GLP/GEP Yes unpublished	N	ISK
Annex III Data and Information					
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Annex I: Summary of trial information

Report No. Location	Commodity/ Variety	Date of 1. Sowing of planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Report No. **RJ1023B** Residue Summary Sheet: FZM/SOLTU 09/D/90 **Germany**											
Büchendorf, D-2059	Potatoes/ Hansa	1) 06.04.90 2) 30.06.90 3) 24.08.90 31.08.90 07.09.90	0.200	400	0.050	9/ 24.08.90	85	tubers	<0.01 <u>≤0.01</u> <0.01	0 7 14	Stage 85 stage 92 stage 95. Mean external standard recovery: 82%. LOQ 0,01 mg/kg
Gödenstorf, D-2125	Potatoes/ Cilena	1) 10.04.90 2) 01.07.90 3) 16.08.90 23.08.90 30.08.90	0.200	400	0.050	8/ 16.08.90	85	tubers	<0.01 <u>≤0.01</u> <0.01	0 7 14	Stage 85 stage 92 stage 95. Mean external standard recovery: 82%. LOQ 0,01 mg/kg
Neufahrn, D-8056	Potatoes/ Rebecca	1) 30.04.90 2) 01.07.90 3) 26.09.90 03.10.90	0.200	400	0.050	10/ 26.09.90	85	tubers	<0.01 <u>≤0.01</u>	0 7	Stage 85 stage 87 Mean external standard recovery: 82%. LOQ 0,01 mg/kg

Report No. Location	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Etzlberg, D-8899 Gachenbach	Potatoes/ Agria	1) 18.04.90 2) 06.07.90 3) 06.09.90 13.09.90	0.200	400	0.050	9/ 26.09.90	83-85	tubers	<0.01 <u>0.01</u>	0 7	Stage 83-85 stage 85 Mean external standard recovery: 82%. LOQ 0,01 mg/kg
Report No. **RJ1025B** Residue Summary Sheet: FZM/SOLTU 08/D/89 **Germany**											
Büchendorf, D-2059	Ware Potatoes/ Christa	1) 23.03.89 2) 01.07.89 3) 08.08.89 14.08.89 21.08.89	0.200	400	0.050	8/ 08.08.89	5 cm	tubers	<0.01 <u>0.01</u> <0.01	0 6 13	Stage 89 stage 95 stage 98. Mean recovery: 89%. LOQ 0,01 mg/kg
Varendorf, D-3116	Ware Potatoes/ Produzent	1) 30.03.89 2) 01.07.89 3) 22.08.89 29.08.89 05.09.89	0.200	400	0.050	10/ 22.08.89	30 cm	tubers	<0.01 <u>0.01</u> <0.01	0 7 14	Stage 81 stage 85 stage 91. Mean recovery: 89%. LOQ 0,01 mg/kg
Offenbach, D-6745	Ware Potatoes/ Christa	1) 08.05.89 2) 28.06.89 3) 15.08.89 22.08.89 29.08.89	0.200	400	0.050	8/ 15.08.89	20 - 30 cm	tubers	<0.01 <u>0.01</u> <0.01	0 7 14	Stage 85/87 stage 89 stage 91. Mean recovery: 89%. LOQ 0,01 mg/kg

Report No. Location	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Neufahrn, D-8056	Ware Potatoes/ Indira	1) 15.04.89 2) June/July 89 3) 07.09.89 14.09.89	0.200	400	0.050	10/ 07.09.89	30 cm	tubers	<0.01 <u><0.01</u>	0 7	Stage 83/85 stage 85 Mean recovery: 89%. LOQ 0,01 mg/kg
Report No. **RJ2308B** Residue Summary Sheet: FZM/SOLTU 30/GB/97 **United Kingdom**											
Melbourne, Derbyshire, GB	Early Potatoes/ Maris Bard	1) 16.04.96 2) 20.06.96 3) 16.08.96	0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200	293 295 295 301 292 309 307 288 295 289	0.068 0.068 0.068 0.066 0.068 0.065 0.065 0.069 0.068 0.069	10/ 09.08.96	92 BBCH	tubers	<u><0.01</u>	7	Mean recovery: 86%. LOQ 0,01 mg/kg

Report No. Location	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Booningale, Shropshire, GB	Early Potatoes/ Pentland Javelin	1) 16.04.96 2) 20.06.96 3) 16.08.96	0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200	205 320 297 297 298 292 305 299 307	0.066 0.063 0.067 0.067 0.067 0.068 0.066 0.067 0.065	9/ 25.07.96	93 BBCH	tubers	0.01	7	Mean recovery: 86%. LOQ 0,01 mg/kg
Report No. **RJ1161B** Residue Summary Sheet: FZM/SOLTU 29/GB/89 **United Kingdom**											
Holbeach, Lincolnshire, GB	Ware Potatoes/ Cara	1) 11.05.89 2) not reported 3) 16.10.89	0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200	188 250 250 312.5 312.5 312.5 312.5 312.5 312.5 312.5	0.106 0.080 0.080 0.064 0.064 0.064 0.064 0.064 0.064 0.064	11/ 09.10.89	09.10.89	tubers	<0.01	7	Recovery (fortification level: 0.02 mg/kg): 79%. LOQ 0,01 mg/kg

Report No. Location	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Holbeach, Lincolnshire, GB	Ware Potatoes/ Romano	1) 10.05.89 2) not reported 3) 25.09.89	0.294 0.200 0.200 0.200 0.200 0.200 0.200 0.200	275 250 250 312.5 312.5 312.5 312.5 312.5	0.106 0.080 0.080 0.064 0.064 0.064 0.064 0.064	8/ 18.09.89	09.10.89	tubers	<0.01	7	Recovery (fortification level: 0.02 mg/kg): 79%. LOQ 0,01 mg/kg
Holme Fen, Peterborough, GB	Ware Potatoes/ Maris Piper	1) 10.05.89 2) not reported 3) 09.10.89	0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200 0.200	250 250 312.5 312.5 312.5 312.5 312.5 312.5 312.5	0.080 0.080 0.064 0.064 0.064 0.064 0.064 0.064 0.064	9/ 02.10.89	02.10.89	tubers	<0.01	7	Recovery (fortification level: 0.02 mg/kg): 79%. LOQ 0,01 mg/kg
Report No. **6530-96-0020-CR-001** Residue Summary Sheet: FZM/SOLTU 15/GR/95 **Greece**											
Thiva, Viotia, GR	Ware Potatoes/ Spunda	1) 15.02.95 2) not reported 3) 13.07.95	0.249 0.256 0.248 0.256 0.259 0.257	990 1017 987 996 1003 995	0.025 0.025 0.025 0.025 0.026 0.026	6/ 28.06.95	20-30 cm plant tallness	tubers	<0.01	15	Mean Recovery: 96%. LOQ 0,01 mg/kg

Report No. Location	Commodity/ Variety	Date of 1. Sowing or planting 2. Flowering 3. Harvest	Application rate per treatment			Dates of treatment(s) or no. of treatment and last date	Growth stage at last treatment or date	Portion analysed	Residues [mg/kg]*	PHI [days]	Remarks
			kg a.i./ha	water L/ha	kg a.i./hL						
Report No. **6953-97-0087-CR-001** Residue Summary Sheet: FZM/SOLTU 21/GR/96 **Greece**											
Thiva, Viotia, GR	Ware Potatoes/ Spunta	1) 20.02.96 2) not reported 3) 10.07.96	0.252 0.247 0.246 0.245 0.247 0.252	707 692 688 785 790 1007	0.036 0.036 0.036 0.031 0.031 0.025	6/ 26.06.96	26.06.96	tubers	<0.01	14	Mean Recovery: 98%. LOQ 0,01 mg/kg
Kokkinoporos, Kagadiou, Achai, GR	Ware Potatoes/ Lizeta	1) 17.08.96 2) not reported 3) 26.11.96	0.251 0.250 0.251 0.256 0.266 0.253	703 700 803 920 1063 1010	0.036 0.036 0.036 0.031 0.031 0.025	6/ 12.11.96	12.11.96	tubers	<0.01*	14	Mean Recovery: 98%. LOQ 0,01 mg/kg * results from analysis of retain samples of same plots on request of sponsor, as the results of the primary samples were 0.01 mg/kg.

* Residues expressed in mg fluazinam /kg