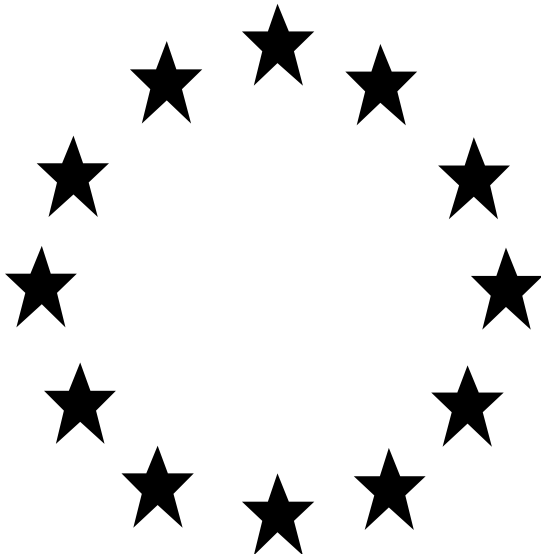


Draft Assessment Report (DAR)

- 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.1 – B. 5

July 2006

Federal Office for Food Safety

Spargelfeldstraße 191
1220 Vienna
Austria

Monograph prepared in the context of inclusion of following active substance in Annex I of the Council Directive 91/414/EEC

Fluazinam

Volume 3

Annex B

Summary, Scientific Evaluation and Assessment

Draft: December 2005

Contents

Annex B

Fluazinam

B.1 Identity

B.1 Identity

B.1.1 Identity of the active substance (Annex IIA 1)

B.1.1.1 Name and address of applicant(s) for inclusion of the active substance in Annex I (Annex IIA 1.1)

Company:

ISK Biosciences Europe S.A.

Avenue Louise 480, B.12
B-1050 Brussels BELGIUM

Contact person:

[redacted]

B.1.1.2 Common name and synonyms (Annex IIA 1.3)

Fluazinam

B.1.1.3 Chemical name (IUPAC and CA nomenclature) (Annex IIA 1.4)

IUPAC: 3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- α,α,α- trifluoro-2, 6-dinitro-p-toluidine

CA: 3-chloro-N-[3-chloro-2, 6-dinitro-4-trifluoromethyl) phenyl]-5-(trifluoromethyl)-2-pyridinamine

B.1.1.4 Manufacturer's development code number (Annex IIA 1.5)

IKF-1216, B-1216, PP192

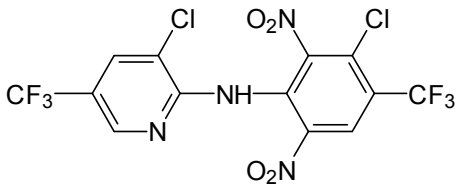
B.1.1.5 CAS, EC (EEC) and CIPAC numbers (Annex IIA 1.6)

CAS:	79622-59-6
EEC:	Not available
CIPAC:	521

B.1.1.6 Molecular and structural formula, molecular mass (Annex IIA 1.7)

Molecular formula: $C_{13}H_4Cl_2F_6N_4O_4$

Structural formula:



Molecular mass: 465.1

B.1.1.7 Manufacturer of the active substance (Annex IIA 1.2)

Confidential information, see Volume 4, Annex C.

B.1.1.8 Method or methods of manufacture (Annex IIA 1.8)

Confidential information, see Volume 4, Annex C.

B.1.1.9 Specification of the purity of the active substance (Annex IIA 1.9)

Reference: Tetsuya Okabyashi (2004): Certificate of Composition of Fluazinam

Guideline: --

GLP: no

Minimum content of fluazinam technical material: 960 g/kg

For detailed information, please refer to Volume 4, Annex C.

B.1.1.10 Identity of inactive isomers, impurities and additives (Annex IIA 1.10)

B.1.1.11 Analytical profile of batches (Annex IIA 1.11)

B.1.1.11.1 Analytical profile of batches of technical active substance

Confidential information, see Volume 4, Annex C (C1.2.3).

B.1.1.11.2 Results of analyses of batches used in toxicological and ecotoxicological tests

The analytical profile of batches used in toxicological testing is included together with all other confidential information in Volume 4, Annex C (C1.2.3.2).

B.1.2 Identity of the plant protection product (Annex IIIA 1)

B.1.2.1 Trade name and manufacturer code number for the preparation

Trade names:

Ohayo (Belgium, Denmark, France, Germany, Greece, Italy and the Netherlands); Legacy (UK); Shirlan (Belgium, Denmark, Estonia, Finland, France, Germany, Ireland, Latvia, Lithuania, Portugal, Sweden, the Netherlands and UK); Shirlan Programme (UK); Altima (Eastern European countries) Sagiterre (France); Salvo (UK); Frowncide (France and Ireland); Winner (Austria)

Code:

Fluazinam 500SC

B.1.2.2 Manufacturer(s) of the preparations (Annex IIIA 1.2)

Confidential information, see Volume 4, Annex C (C.1.1.1).

B.1.2.3 Type of preparation and code (Annex IIIA 1.5)

Suspension concentrate (SC)

B.1.2.4 Function (Annex IIA 3.1, Annex IIIA 1.6)

Fungicide

B.1.2.5 Composition of the preparations (Annex IIIA 1.4)

B.1.2.5.1 Contents of active substance(s) and formulants

Reference: ISK (2005): Composition Report N° IBE1216-CI0506-02

Content of fluazinam (IKF-1216): 500.0 g/L

Information with respect to formulants is included with all other confidential information in Volume 4, Annex C (C1.3.2).

B.1.2.5.2 Salt, ester, anion or cation present for each active substance

Not relevant as fluazinam does not form salts, ester, anions or cations.

B.1.3. References relied on

See Volume 4 Annex C

Annex B

Fluazinam

B.2 Physical and chemical properties

B. 2 Physical and chemical properties

B.2.1 Physical and chemical properties of the active substance (Annex IIA 2)

Table B.2.1-1 Summary of the physical and chemical properties of the active substance

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	EEC/A1 (Differential scanning calorimetric method) GLP	Purified product (purity: 99.8% w/w) Melting point: 117 °C	Acceptable	van Helvoirt, J.A.M.W. (1993) (Document 089033)
B.2.1.2 Boiling point (IIA 2.1.2)	Statement	Material is solid and does not have a low melting point	Acceptable	van Helvoirt, J.A.M.W. (1993) (Document 089044)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)			Not relevant as the melting point was determined	
B.2.1.4 Relative density (IIA 2.2)	EEC/A3 (Gas comparison pycnometer) GLP	Purified product (purity: 99.8% w/w) D_4^{20} = 1.81 20.0 ± 1.0 °C	Acceptable	van Rijsbergen, L.M. (2002) (Document 341123)
B.2.1.5 Vapour pressure (IIA 2.3.1)	EEC/A4 GLP	Purified product (purity: 99.8% w/w) (7.5 ± 0.8) x 10^{-3} Pa at 20 °C The vapour pressure at 20 °C was extrapolated from the vapour pressure curve.	Acceptable	van Rijsbergen, L.M. (2002) (Document 341134)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)		25.9 $Pa.m^3.mol^{-1}$ (20 °C) values used for calculation: water solubility: 1.35 x 10^{-4} g/L at pH 7 and 20 °C vapour pressure: (7.5 ± 0.8) x 10^{-3} Pa at 20 °C	Acceptable	McFadden, J.J. (2000) (Document F-150-A))
B.2.1.7 Appearance: physical state (IIA 2.4.1)	Visual examination	Purified product (purity: 100% w/w) crystalline solid		Kimura, T. (1991) (Document 91 0508KT)
	Visual examination	Technical product (purity: 97.7% w/w) solid		Asai, N. (1991) (Document 1216-90-06303-1)
B.2.1.8 Appearance: colour (IIA 2.4.1)	Visual examination	Purified product (purity: 100% w/w) Munsell color = 2.5GY 9/8 (yellow)		Kimura, T. (1991) (Document 91 0509KT)
	Visual examination	Technical product (purity: 97.7% w/w) Munsell color = “5Y 9/4” or “5Y/5” (yellow)		Oguri, M. (1991) (Document 1216-90-06302-1)
B.2.1.9 Appearance: odour (IIA 2.4.2)	Organoleptic examination	Purified product (purity: 99.1% and 100% w/w) odorless at 20 – 22 °C		Kimura, T. (1991) (Document 91 0510KT)
	Organoleptic examination	Technical product (purity: 97.7% w/w) weak aromatic hydrocarbon-like at 23 – 24 °C		Asai, N. (1991) (Document 1216-90-06304-1)

<table>
<tr><th>Study</th><th>Method</th><th>Results</th><th>Conclusion/Comment</th><th>Reference</th></tr>
<tr><td rowspan="3">B.2.1.10
Spectra of the active substance
(IIA 2.5.1)</td><td>UV/VIS - Spectroscopy
OECD guideline No.101
GLP</td><td>Purified product (purity: 99.8% w/w) c = 4.66 x 10^{-5} mol/L
<table><tr><th>Solvent</th><th>$\lambda_{max\ [nm]}$</th><th>ε_{max} [L·mol^{-1}·cm^{-1}]</th></tr><tr><td>MeOH/HCl
[90/10 (0.1 N) v/v]</td><td>238</td><td>21900</td></tr><tr><td>MeOH</td><td>238</td><td>21200</td></tr><tr><td>MeOH/NaOH
[90/10 (0.1 N) v/v]</td><td>260
341
479</td><td>18100
20100
3710</td></tr></table>ε above 290 nm in alkaline solution > 10</td><td>The UV spectra show in neutral and acidic media additional absorbance at approx. 340 nm, which is not reported. Data requirement see volume 1 level 4.</td><td>van Rijsbergen, L.M. (2002)
(Document 341167)</td></tr>
<tr><td>US EPA Product Properties Tst Guidelines OPPTS 830.7050
GLP</td><td>Purified product (purity: 99.7% w/w) c = 4.66 x 10^{-5} mol/L
<table><tr><th>pH</th><th>$\lambda_{max\ [nm]}$</th><th>ε_{max} [L·mol^{-1}·cm^{-1}]</th></tr><tr><td>< 2</td><td>238</td><td>20615</td></tr><tr><td>7 ± 0.2</td><td>239
342</td><td>18588
7251</td></tr><tr><td>> 10</td><td>260
343
482</td><td>16663
18619
3439</td></tr></table>ε above 290 nm in neutral and alkaline solution > 10</td><td>The UV spectrum shows in acidic medium additional absorbance at approx. 350 nm, which is not reported.</td><td>Gallacher, A.C. (1997)
(Document 4039-97-0017-AS-001)</td></tr>
<tr><td>FTIR - Spectroscopy
KBr disk,
400 – 4000 cm^{-1}
GLP</td><td>Purified product (purity: 99.8%)</td><td>Acceptable
The IR spectrum of fluazinam is in agreement with the chemical structure</td><td>van Rijsbergen, L.M. (2002)
(Document 341145)</td></tr>
</table>

Study	Method	Results	Conclusion/Comment	Reference
	Fourier–Transform ^{1}H - NMR-Spectroscopy GLP	Purified product (purity: 99.8% w/w)	Acceptable The NMR spectrum of fluazinam is in agreement with the chemical structure	van Rijsbergen, L.M. (2002) (Document 341156)
	MS - Spectroscopy MS/MS (API negative mode) GLP	Purified product (purity: 99.8% w/w) Additional to the molecular mass spectrum, spectra with different collision energy settings (-20 and -88 V) to induce fragmentation are performed	Acceptable The MS-spectrum is consistent with the chemical structure	van Rijsbergen, L.M. (2002) (Document 341178)
B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)	MS (EI and CI), IR, ^{13}C - NMR and UV spectrum GLP	<u>Impurity 5:</u> Purity 97.3% Concentration: 0.45 mg/mL in acetonitrile UV: $\lambda_{max\ [nm]}$: 239; ε_{max} [L·mol^{-1}·cm^{-1}]: 18893	MS, IR and NMR spectra confirm the structure of impurity 5. The UV spectrum shows an additional absorbance at approx. 297 nm, which is not reported. Data requirement see volume 1 level 4	Bramstedt W.R., Kogovsek L.M. (1999) (Document 4039-98-0177-AS-001)
		<u>Impurity 6:</u>	Spectra are missing. Data requirement see volume 1 level 4	
B.2.1.12 Solubility in water (IIA 2.6)	EEC/A6 column elution method GLP	Purified product (purity: 99.8% w/w) at 20 ± 1 °C 1.06 x 10^{-4} g/L in buffered solution (at pH 5) 1.35 x 10^{-4} g/L in buffered solution (at pH 7) 2.72 x 10^{-3} g/L in buffered solution (at pH 9)	Acceptable	Brekelmans, M.J.C. (2002) (Document 341189)

Study	Method	Results		Conclusion/Comment	Reference
B.2.1.13 Solubility in organic solvents (IIA 2.7)	in house method (HPLC and GC) GLP	Technical product (purity: 96.8% w/w)		Acceptable	Sanders, J. (1993) (Document 4039-91-0384-AS-001)
		solvent	solubility at 25 °C [g/L]		
		acetone	853		
		dichloromethane	675		
		ethyl acetate	722		
		ethyl ether	231		
		hexane	8		
		methanol	192		
		octanol	41		
		toluene	451		
B.2.1.14 Partition coefficient n-octanol/water (IIA 2.8)	40 CFR 158.190 Pesticide Assessment Guidelines Subdivision D: Product Chemistry Guideline 63-11 GLP	Technical product (purity: 96.8% w/w) K_{ow} = 1.08 x 10^4 log K_{ow} = 4.03 neutral range at 25 °C		The method is comparable to the EEC/A8 shake flask method	Sanders, J. (1992) (Document 4039-91-0386-AS-001)
	OECD 122 Draft (Partition coefficient, pH-metric method for ionisable substances) calculation of the log P_{OW} value as a function of pH	The model calculation (graph) for fluazinam (weak acid) in its non-dissociated form shows an octanol/water coefficient of 4.19 (pH 4 to 7) 3.5 (pH 8) 2.5 (pH 9)		Acceptable	De Smet B. (2005) (Document IBE1216-PC0507-02)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.15 Hydrolysis rate (IIA 2.9.1)	OECD 111 EEC/C7 EPA OPPTS 835.2110, SETAC (Europe) Procedures for assessing the environmental fate and ecotoxicity of pesticides Part 9 Aqueous Hydrolysis GLP	Purified product (purity: 99.8% w/w) unlabelled, [^{14}C-phenyl] Fluazinam (2.33 GBq mmol^{-1}, 100% radiopurity) DT_{50} (25 °C): stable at pH 4 DT_{50} (25 °C): 4.5 d at pH 7 DT_{50} (25 °C): 3.5 d at pH 9 [^{14}C-pyridyl] Fluazinam (2.37 GBq mmol^{-1}, 97.7% radiopurity) DT_{50} (25 °C): stable at pH 4 DT_{50} (25 °C): 2.7 d at pH 7 DT_{50} (25 °C): 3.9 d at pH 9 Fluazinam may be considered hydrolytic stable under acidic conditions, under neutral and alkaline conditions it is rapidly hydrolysed <u>Degradation products:</u> CAPA (5-chloro-6-(3-chloro- α,α,α-trifluoro-2,6-dinitro-p-toluidino)-nicotinic acid), which is then steadily degraded to DCPA (6-(4-Carboxy-3-chloro-2,6-dinitroanilino)-5-chloronicotinic acid	Acceptable For details see B 8.4 Fate and behaviour in water	van der Gaauw, A. (2003) (Document 846211)
B.2.1.16 Direct phototrans-formation (IIA 2.9.2)	United States EPA Guideline 161-2 EC Directive, Annex II, Sections 2.9.2 and 7.2.1.2 GLP	Purified product (purity: 99.6% w/w) unlabelled [^{14}C-phenyl] IKF-1216 (57.3 mCi/ mmol, >99%) [^{14}C-pyridyl] IKF-1216 (66.2 mCi/ mmol, >99%) DT_{50} = 2.5 days in sterile buffer (pH 5 ± 0.05) for both labels at 25 ± 1 °C One major photolyte was detected for both labels and accounted for 17.1% and 14.0% of the phenyl and pyridyl labels, at day 10 and 7, respectively. It was identified as 4,9-dichloro-6-nitro-8-(trifluoromethyl)pyrido[1,2-α]benz- imidazole-2-carboxylic acid. The major photolytic product was $^{14}CO_2$ (17.7% and 16.0% of the phenyl and pyridyl labels, respectively after 30 days)	Acceptable For details see B 8.4 Fate and behaviour in water	Lentz, N.R., Korsch, B.H. (1995) (Document 5312-94-0119-EF-002)
B.2.1.17 Quantum yield (IIA 2.9.3)	Calculation	Quantum yield in moles degraded per Einstein absorbed 5.1×10^{-5} (pH 5 buffer) 1.7×10^{-5} (pH 6 distilled water) 2.1×10^{-6} (pH 9 buffer)	Acceptable For details see B 8.4 Fate and behaviour in water	Wadley, A.M. (1992) (Document RIC1726)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.4)	40 CFR 158.190 Pesticide Assessment Guidelines, Subdivision D: Product Chemistry Guideline 63-10 UV spectrophotometric method.	Purified product (purity: 99.9% w/w) pK_A = 7.34 (20 ± 1 °C)	Acceptable The submitted method is comparable to OECD 112	Gallacher, A.C. (1992) (Document 4039-91-0387-AS-001)
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Atkinson calculation	Estimate of overall reaction rate constant with hydroxyl radicals is between 6.1 x 10^{-11} and 1.5 x 10^{-12} $cm^3molecule^{-1}sec^{-1}$ t½ : 2.8 hours to approximately 10 days (using 12-hour exposure period) According Section Fate and behaviour the substance is stable in the troposphere (DT_{50} > 2 days)	Recalculation by RMS using computer program AOPWIN vers. 1.91. assuming a 12 hour daytime cycle and an OH concentration of 1.5 x 10^6 molecules/cm^3 the calculated half-life of fluazinam was **163 days**. For details see B 8.7.1 Fate and behaviour in air	Atkinson, R. (1993) (Document RIC1832)
B.2.1.20 Flammability (IIA 2.11)	EEC/A10 GLP	Technical product (purity: 96.7% w/w) Preliminary test: The test substance could not be ignited by a flame. Emission of yellow sparks with the ignition source. After removal of the ignition source, no more sparks were observed. According to EEC/A10 no further testing is required.	Acceptable Technical fluazinam is not considered as “highly flammable” under test condition	van Rijsbergen, L.M. (2002) (Document 341191)
B.2.1.21 Auto-flammability (IIA 2.11.2)	EEC/A16 GLP	Technical product (purity: 96.7% w/w) No self ignition up to 400 °C	Acceptable Compound is not considered as auto-flammable under test condition	van Rijsbergen, L.M. (2002) (Document 341202)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.22 Flash point (IIA 2.12)			Not applicable as the melting point is > 40 °C	
B.2.1.23 Explosive properties (IIA 2.13)	EEC/A14 GLP	Technical product (purity: 97.8% w/w) Thermal sensitivity test: no explosion after 5 minutes (nozzle diameter: 2.0 mm) Shock test: no explosion occurred within 6 tests using a mass of 10 kg from a height of 0.4 m Friction test: no explosion occurred within 6 tests using a 360 N loading	Acceptable Technical fluazinam does not present a danger of explosion under test condition	Angly H. (2005) (Document 2005.4004.EXP)
B.2.1.24 Surface tension (IIA 2.14)	EEC/A5 Ring method GLP	Technical product (purity: 95.5% w/w) σ = 66.3 mN/m at 20 ± 0.5 °C (90% of a saturated solution in water)	Acceptable The compound is not surface active	van Rijsbergen, L.M. (2002) (Document 341213)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.25 Oxidising properties (IIA 2.15)	Statement according to EEC/A17	Technical product (purity: 97.8% w/w) The notifier states that the two NO_2- groups are relatively small groups in the molecule of fluazinam and therefore it is not expected that fluazinam will function as an oxidizing reagent. This because of the deficiency of oxygen; the test substance will not supply enough oxygen atoms to propagate any burning reaction. Fluazinam contains the electronegative atoms oxygen, fluoride and chloride. Fluoride and chloride are bound to carbon and therefore are not regarded as oxidizing. Oxygen, however, is bound to another electronegative element (nitrogen).	According to *Recommendations on the transport of dangerous goods, Manual of tests and criteria, United Nations (appendix 6):* Oxygen must be chemically bonded only to carbon or hydrogen that the screening procedure (statement) can be applied. In this case oxygen is bound to nitrogen. Therefore a test according to method EEC/A17 must be submitted. Data requirement see volume 1 level 4	Van der Baan - Treur J. (2005) (Document 435072)

B.2.2 Physical, chemical and technical properties of the plant protection product (Annex IIIA 2)

B.2.2.1 Fluazinam 500 SC suspension concentrate, 500 g/L

Table B.2.2.1-1 Summary of the physical and chemical properties of the plant protection product

Study	Method	Results	Comment	Reference
B.2.2.1 Appearance: physical state (IIIA 2.1)	40 CFR 158.190 Pesticide Assessment Guidelines Subdivision D: Product Chemistry Guideline 63-2, 3, 4 Color, Physical State, Odor GLP	Homogeneous suspension		van Rijsbergen, L. M. (2002) (Document 341224)
B.2.2.2 Appearance: colour (IIIA 2.1)	40 CFR 158.190 Pesticide Assessment Guidelines Subdivision D: Product Chemistry Guideline 63-2, 3, 4 Color, Physical State, Odor GLP	light-yellow		van Rijsbergen, L. M. (2002) (Document 341224)
B.2.2.3 Appearance: odour (IIIA 2.1)	40 CFR 158.190 Pesticide Assessment Guidelines Subdivision D: Product Chemistry Guideline 63-2, 3, 4 Color, Physical State, Odor GLP	no characteristic odor		van Rijsbergen, L. M. (2002) (Document 341224)

Study	Method	Results	Comment	Reference
B.2.2.4 Explosive properties (IIIA 2.2.1)	Justification	The active substance shows no explosive properties and all other formulants are not estimated to be explosive.	Acceptable	Widmer H. (2005) (Document 22904)
B.2.2.5 Oxidising properties (IIIA 2.2.2)	Expert Statement	The notifier stated that according to EEC method A17 the active compound fluazinam is non-oxidizing. An assessment of the oxidizing property of each formulant is reported.	The justification cannot be accepted as a study according to **EEC/A17 for the active substance** is required (see B.2.1.25 Oxidising properties) Data requirement see volume 1 level 4	De Smet B. (2005) (Document IBE1216-PC0507-01)
B.2.2.6 Flash point (IIIA 2.3)	Justification	Test substance melts above 40 ºC	The justification cannot be accepted as the requirement for the determination of the flash point of liquids is the existence of flammable solvents in the formulation, but due to the fact that Fluazinam 500 SC is a water based formulation the performance of the study is not necessary	
B.2.2.7 Flammability (IIIA 2.3)	Justification	Formulation is an aqueous suspension	Not relevant for liquid formulations	

Study	Method	Results	Comment	Reference
B.2.2.8 Auto-flammability (IIIA 2.3)	EEC/A15 GLP	No self ignition in the temperature range of 200 ºC to 650 ºC (at atmospheric pressure: 1031.8 hPa)	Acceptable The test substance is not considered as auto-flammable under test condition	van Rijsbergen, L. M. (2002) (Document 341235)
B.2.2.9 Acidity or alkalinity and pH value (IIIA 2.4.1)	CIPAC MT 75 GLP	pH = 7.63 (neat formulation) Preparation is not acidic (pH <4) or alkaline (pH> 10)	Acceptable	Bird, N.R. (1993) (Document RY0013B)
B.2.2.10 pH of 1 % aqueous dilution, emulsion or dispersion (IIIA 2.4.2)	CIPAC MT 75 GLP	pH = 6.56 (1% v/v dispersion in deionised water) at ambient temperature	Acceptable	Bird, N.R. (1993) (Document RY0013B)
	CIPAC MT 75 GLP	pH = 5.4 (1% w/v solution in deionised water) at ambient temperature	Acceptable	Stanley, R.D., Johnson, P.S., (1992) (Document BL4535/B)
B.2.2.11 Viscosity (IIIA 2.5)	OECD 114 GLP	123 mPas (using a shear rate of 64.5 s^{-1}) and 61.8 mPas (using a shear rate of 258 s^{-1}) at 20 °C 92.8 mPas (using a shear rate of 64.5 s^{-1}) and 431.8 mPas (using a shear rate of 258 s^{-1}) at 40 °C	Acceptable	Stanley, R.D., Johnson, P.S., (1992) (Document BL4535/B
B.2.2.12 Surface tension (IIIA 2.5)	EEC/A5 GLP	The surface tension is 53.1 mN/m at 20 °C of a solution of the preparation in water at a 90% saturated concentration	Acceptable	van Rijsbergen, L. M. (2002) (Document 341246)
B.2.2.13 Relative density (IIIA 2.6.1)	CIPAC MT 3.2 GLP	Density is 1.29 g/cm^3 at 20 ºC (relative density is not reported)	Acceptable	Bird, N.R. (1993) (Document RY0013B)
	EEC/A3 pycnometer method GLP	Density is 1.228 g/cm^3 at ambient temperature (relative density is not reported)		Stanley, R.D., Johnson, P.S., (1992) (Document BL4535/B)

Study	Method	Results			Comment	Reference
B.2.2.14 Bulk or tap density (IIIA 2.6.2)					Not relevant for liquid formulation	
B.2.2.15 Stability after storage for 14 days at 54 °C (IIIA 2.7.1)	CIPAC MT 46 GLP	**Test**	**initial**	**after 14 days at 54 °C**		Bird, N.R., Swaine, H. (1993) (Document RY0003B)
	[1] 1L PET bottle	Visual estimation		The seal was in good condition but did show very slight signs of reaction with the formulation	Acceptable	
	[2] 5L HDPE bottle	Visual estimation		Some slight top panelling but an intact seal was observed	Acceptable	
	HPLC	content a.i.	38.1% w/w	38.1% w/w [1] 37.1% w/w [2]	Acceptable	
	Visual estimation	Phys. state	yellow liquid	yellow liquid	Acceptable	
	CIPAC MT 75	pH neat formulation	7.63	8.72 [1] 8.74 [2]	Acceptable	
	CIPAC MT 160	Spontaneity dispersion	96.7%	103.7% [1] 101.2% [2]	Acceptable	
	CIPAC MT 47.2	Persistent foaming (max. appl. Rate)	24 mL after 1 min	24 mL [1] 32 mL [2] after 1 min	Acceptable	
	CIPAC MT 59.3	Wet sieving residue on 75 µm sieve	0.020%	0.006% [1] 0.009% [2]	Acceptable	
	CIPAC MT 161	Suspensibility (n = 2)	Low application concentration (0.1%)		Acceptable	
			93.5%	87.6% [1] 86.1% [2]		
			High application concentration (1.6%)			

Study	Method	Results			Comment	Reference
			91.4%	94.0% [1] 94.8% [2]		
Stability after storage for 8 weeks at 40 °C	CIPAC MT 46 GLP	**Test**	**initial**	**after 8 weeks at 40 °C**		Bird, N.R. (1993) (Document RY0013B)
	[1] 1L PET bottle	visual		The seal was in good condition	Acceptable	
	[2] 5L HDPE bottle	visual		Some slight panelling on the top but an intact seal was observed	Acceptable	
	HPLC	content a.i.	38.1% w/w	41.0% w/w [1] 39.4% w/w [2]	Acceptable	
	CIPAC MT 75	pH (neat formulation)	7.63	8.58 [1] 8.61 [2]	Acceptable	
		pH (1%)	6.56 pH of test water = 5.71	Not reported		
	CIPAC MT 160	Spontaneity dispersion	96.7%	108.6% [1] 101.8% [2]	Acceptable	
	CIPAC MT 47.2	Persistent foaming (in mL) max. application rate	24 after 1 min	32 [1] 30 [2] after 1 min	Acceptable	
	CIPAC MT 59.3	Wet sieving residue on 75 µm sieve	0.020%	0.04% [1] 0.06% [2]	Acceptable	
	CIPAC MT 161	Suspensibility (n = 2)	Low application concentration (0.1%)		Acceptable	
			93.5%	104.4% [1] 97.2% [2]		
			High application concentration (1.6%)			

Study	Method	Results			Comment	Reference
			91.4%	95.8% [1] 94.8% [2]		
B.2.2.16 Effect of low temperature on stability (IIIA 2.7.2)	CIPAC MT 39.3 GLP	No separated material was observed at the end of the test			Acceptable	Schiffers, B. (2002) (Document 7-FLUAZINA 02/01)
		Test	**initial**	**after 7 days**		
	HPLC	content a.i.	39.4% w/w	39.1% w/w	Acceptable	
	CIPAC MT 59.3	Wet sieving residue on 75 µm sieve	0.017%	0.039%	Acceptable	
	CIPAC MT 161 chemically	Suspensibility (n = 2)	Low application concentration (0.1%)		Acceptable	
			98%	98%		
			High application concentration (1.6%)			
			98%	98%		

Study	Method	Results				Comment	Reference
B.2.2.17 Shelf life (IIIA 2.7.3)	GLP [1] 1L PET bottle [2] 5L HDPE bottle					No major changes after 2 years of storage. According to the FAO spec. for SC formulations the content of the relevant impurities and the pourability before and after storage must be determined. Data requirement see volume 1 level 4	Bird, N.R., Swaine, H. (1995) (Document RY0072B)
		Test	**initial**	**after 24 wks at 40 °C**	**after 104 wks at 25°C**		
	HPLC	content a.i.	38.1% w/w	40.2% w/w[1] 39.5% w/w[2]	42.8% w/w [1] 40.5% w/w [2]	Acceptable	
	Visual estimation	appearance	uniform, opaque, mobile, yellow liquid	No significant change [1, 2]	No significant change [1, 2]	Acceptable	
	CIPAC MT 75.1	pH neat formulation	7.63	8.56[1] 8.63[2]	8.44 [1] 8.53 [2]	Acceptable	
	CIPAC MT 160	Spontaneity dispersion	96.7%	100.7%[1] 100.6%[2]	95.3% [1] 95.8% [2]	Acceptable	
	CIPAC MT 47.2	Persistent foaming (in mL) max. appl. rate	23.7 after 1 min	24 [1] 30 [2] after 1 min	22.2 [1] 23.4 [2] after 1 min	Acceptable	
	CIPAC MT 59.3	Wet sieving residue on 75 µm sieve	0.02%	0.14%[1] 0.57%[2]	0.004% [1] 0.02% [2]	Acceptable	
	CIPAC MT 161	Suspensibi	Low application concentration (0.1%)			Acceptable	

Study	Method	Results				Comment	Reference
	chemically	lity (n = 2)	93.5%	91.1%[1] 89.6%[2]	90.5% [1] 91.9% [2]		
			High application concentration (1.6%)			Acceptable	
			91.4%	90.5%[1] 93.6%[2]	90.2%[1] 90.5%[2]		
		Surface tension (1%)	Not reported	Not reported	40 mN/m [1] 42 mN/m [2]	Not required	
B.2.2.18 Wettability (IIIA 2.8.1)						Not relevant for liquid formulations	
B.2.2.19 Persistent foaming (IIIA 2.8.2)	CIPAC MT 47.2	< 60 mL after 1 minute concentration: max. application rate (1.6 %)				Acceptable	Bird, N.R., Swaine, H. (1993) (Document RY0003B)
B.2.2.20 Suspensibility (IIIA 2.8.3.1)	CIPAC MT 161 chemically	After 30 minutes at 30 °C (±1 °C) in CIPAC water C (500 mg/kg hardness) the values are 93.5% at low application rate (0.1%) 91.4% at high application rate (1.6%)				Acceptable	Bird, N.R., Swaine, H. (1993) (Document RY0003B)
B.2.2.21 Spontaneity of dispersion (IIIA 2.8.3.2)	CIPAC MT 160 GLP	99% 5% formulation in CIPAC water D at 30 ± 1 °C				Acceptable	van Rijsbergen, L. M. (2002) (Document 341257)
B.2.2.22 Dilution stability (IIIA 2.8.4)						Not relevant as the preparation is not a water soluble product	
B.2.2.23 Dry sieve test (IIIA 2.8.5)						Not relevant for suspension concentrate	

Study	Method	Results	Comment	Reference
B.2.2.24 Wet sieve test (IIIA 2.8.5)	CIPAC MT 59.3	0.020% Wet sieving residue on a 75 µm sieve	Acceptable	Bird, N.R., Swaine, H. (1993) (Document RY0003B)
B.2.2.25 Size distribution of particles - Nominal size range of particles (IIIA 2.8.6.1)			Not relevant for suspension concentrate	
B.2.2.26 Dust content/particle size (IIIA 2.8.6.2)			Not relevant for suspension concentrate	
B.2.2.27 Attrition and friability (IIIA 2.8.6.3)			Not relevant for suspension concentrate	
B.2.2.28 Emulsifiability, re-emulsifiability and emulsion stability (IIIA 2.8.7.1)			Not relevant (preparation does not form emulsions)	
B.2.2.29 Stability of dilute emulsions (IIIA 2.8.7.2)			Not relevant (preparation does not form emulsions)	
B.2.2.30 Flowability (IIIA 2.8.8.1)			Not relevant for suspension concentrate	

Study	Method	Results	Comment	Reference
B.2.2.31 Pourability (rinsability) (IIIA 2.8.8.2)	CIPAC MT 148 GLP	Initial residue = 1.60% Rinsed residue = 0.17%	Acceptable	Bird, N.R. (1993) (Document RY0013B)
B.2.2.32 Dustability (IIIA 2.8.8.3)			Not relevant (preparation is not a dustable powder)	
B.2.2.33 Physical and chemical compatibility of tank mixes (IIIA 2.9)	Physical compatibility: Static laboratory test of the physical compatibility of tank mixtures as devised by the Agronomy and Applications Sub Group (BAA) and approved by the Regulatory Affairs committee (1986)	Fluazinam 500 SC has been shown to be physically compatible with the following products: Ambush C Anvil 5SC Aphox Sportak 45 Dow Shield Karate 5EC Rubigan Compatible with continuous agitation: Bravo 500 Childion	Acceptable	Fletcher, N. (1992) (Document RIC 1778)
	Chemical compatibility: Statement	Fluazinam 500 SC has been used for more than 10 years in Europe and no chemical compatibility problems are known. Mixtures, which are tested for phys. compatibility, are fully satisfactory when used in accordance with the instructions for use of each product.	Acceptable as no method for the determination of chemical compatibility is available	Vermissen C. (2005) (IBE 1216-PC0705-01)
B.2.2.34 Adherence and distribution to seeds (IIIA 2.10)			Not relevant (preparation is not intended for seed treatment)	

B.2.3 Summary of physical and chemical properties

B.2.3.1 Active substance

Fluazinam pure and technical active substance is a yellow solid.
The melting point is 117 °C for the purified substance. The relative density determined at 20 °C is 1.81. The vapour pressure of the active substance is (7.5 ± 0.8) x 10^{-3} Pa at 20 °C. The Henry´s constant is calculated to be 25.86 Pa**.**m³**.**mol^{-1} at pH 7 and 20 °C. The IR-, MS- and NMR-spectra are in agreement with the chemical structure.
Solubility values in water are 0.106 mg/L at pH 5 , 0.135 mg/L at pH 7 and 2.72 mg/L at pH 9, all measured at 20 °C. The test substance is readily soluble in all organic solvents. The log P_{ow} is 4.03 (neutral range and 25 °C), The pKa value is 7.34 at 20 °C.
The active substance is not highly flammable, auto-flammable or explosive. The determination of oxidizing properties is required. The decadic molar extinction coefficient for neutral and acidic media > 290 nm must be reported.

B.2.3.3 Plant protection product

Fluazinam 500SC is a suspension concentrate containing 500 g/L active ingredient. The homogeneous suspension is light-yellow with no characteristic odour. The formulation is not explosive or flammable. Its pH is within the range that occurs naturally, e.g., in soil. Its technical properties indicate that no particular problems are to be expected, when it is used as recommended. Storage stability data indicate that its stability allows storage under practical and normal commercial conditions. The determination of pourability and content of relevant impurities before and after storage are required just as the determination of the oxidizing properties.

B.2.4 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					
IIA, 2.1.1/01	van Helvoirt, J.A.M.W.	1993	Determination of the Melting Point/Melting Range of IKF-1216 (PAI). RCC NOTOX, Report No. 089033 GLP: yes unpublished	N	ISK
IIA, 2.1.2/01	van Helvoirt, J.A.M.W.	1993	On the Determination of the Boiling Point/Boiling Range of IKF-1216 (PAI). RCC NOTOX, Statement No. 089044 GLP: no unpublished	N	ISK
IIA, 2.2/01	van Rijsbergen, L.M.	2002	Determination of the Density of IKF-1216 PAI. Notox B.V., Report No. 341123 GLP: yes unpublished	Y	ISK
IIA, 2.3.1/02	van Rijsbergen, L.M.	2002	Determination of the Vapor Pressure of IKF-1216 PAI. Notox B.V., Report No. 341134 GLP: yes unpublished	Y	ISK
IIA, 2.3.2/01	McFadden, J.J.	2000	Henry's Law Constant for Fluazinam. Ricerca, Inc., Report No. F-150-A GLP: no unpublished	N	ISK
IIA, 2.4.1/01	Kimura, T.	1991	IKF-1216 (Pure Grade) - Determination of Physical State. Ishihara Sangyo Kaisha, Ltd., Report No. 91 0508KT GLP: no unpublished	N	ISK
IIA, 2.4.1/02	Kimura, T.	1991	IKF-1216 (Pure Grade) - Determination of Color. Ishihara Sangyo Kaisha, Ltd., Report No. 91 0509KT GLP: no unpublished	N	ISK
IIA, 2.4.1/03	Oguri, M.	1991	IKF-1216 (Pure Grade) - Determination of Color. Ishihara Sangyo Kaisha, Ltd., Report No. 1216-90-06302-1	N	ISK
IIA, 2.4.1/04	Asai, N.	1991	IKF-1216 (Pure Grade) - Determination of Physical State. Ishihara Sangyo Kaisha, Ltd., Report No.	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
			1216-90-06303-1 GLP: no unpublished		
IIA, 2.4.2/01	Kimura, T.	1991	IKF-1216 (Pure Grade) - Determination of Odor. Ishihara Sangyo Kaisha, Ltd., Report No. 91 0510KT GLP: no unpublished	N	ISK
IIA, 2.4.2/02	Asai, N.	1991	IKF-1216 (Pure Grade) - Determination of Odor. Ishihara Sangyo Kaisha, Ltd., Report No. 1216-90-06304-1 GLP: no unpublished	N	ISK
IIA, 2.5.1/01	Gallacher, A.C.	1997	IKF-1216 - UV-VIS Absorption. Ricerca, Inc., Report No. 4039-97-0017-AS-001 GLP: yes unpublished	N	ISK
IIA, 2.5.1/02	van Rijsbergen, L.M.	2002	Determination of the UV-VIS Absorption Spectra of IKF-1216 PAI. Notox B.V., Report No. 341167 GLP: yes unpublished	Y	ISK
IIA, 2.5.1/03	van Rijsbergen, L.M.	2002	Determination of the 1H NMR Spectrum of IKF-1216 PAI. Notox B.V., Report No. 341156 GLP: yes unpublished	Y	ISK
IIA, 2.5.1/04	van Rijsbergen, L.M.	2002	Determination of the Mass Spectrum of IKF-1216 PAI. Notox B.V., Report No. 341178 GLP: yes unpublished	Y	ISK
IIA, 2.5.1/05	van Rijsbergen, L.M.	2002	Determination of the IR Absorption Spectrum of IKF-1216 PAI. Notox B.V., Report No. 341145 GLP: yes unpublished	Y	ISK
IIA, 2.5.2	Bramstedt, W. R., Kogovsek, L. M	1999	Characterization of B-1457 (IKF-1216 Impurity, Lot 9604). Ricerca, Inc., Report No. 4039-98-0177-AS-001 GLP: yes	Y	ISK
IIA, 2.6/01	Brekelmans, M.J.C.	2002	IKF-1216 PAI, Determination of the Water Solubility at 3 pH Values. Notox B.V., Report No. 341189 GLP: yes	Y	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
			unpublished		
IIA, 2.7/01	Sanders, J.M.	1993	Fluazinam (IKF-1216) (ASC-66825) – Solubility. Ricerca, Inc., Report No. 4039-91-0384-AS-001 GLP: yes unpublished	N	ISK
IIA, 2.8/01	Sanders, J.M.	1992	Fluazinam (IKF-1216) (ASC-66825) - Octanol/Water Partition Coefficient. Ricerca, Inc., Report No. 4039-91-0386-AS-001 GLP: yes unpublished	N	ISK
IIA, 2.8/02	De Smet B.	2005	Determination of the partitioning coefficient (n-Octanol/water) of IKF-1216 at pH 4-10 ISK Biosciences Europe S.A., report no. IBE1216-PC0507-02, July 12, 2005 Not GLP, unpublished	Y	ISK
IIA, 2.9.1/02	van der Gaauw, A.	2003	14C-Fluazinam: Hydrolysis at Three Different pH Values. RCC Ltd, Report No. 846211 GLP: yes unpublished	Y	ISK
IIA, 2.9.2/01	Lentz, N.R., Korsch, B.H.	1995	A Photolysis Study of IKF-1216 (Fluazinam) in Water at pH 5. Ricerca, Inc., Report No. 5312-94-0119-EF-002 GLP: yes unpublished	N	ISK
IIA, 2.9.3/01	Wadley, A.M..	1992	Fluazinam: Quantum Yield Calculation. Zeneca Report No. Not Available GLP: no unpublished	N	ISK
IIA, 2.9.4/01	Gallacher, A.C.	1992	Fluazinam (IKF-1216) (ASC-66825) - Dissociation Constant. Ricerca, Inc., Report No. 4039-91-0387-AS-001 GLP: no unpublished	N	ISK
IIA, 2.10/01	Atkinson, R.	1993	Estimation of Hydroxyl Radical Reaction Rate Constants: Fluazinam. Ricerca Inc., Report No. RIC 1832 GLP: no unpublished	N	ISK
IIA, 2.11.1/01	van Rijsbergen, L.M.	2002	Determination of the Flammability of IKF-1216 TGAI. Notox B.V., Report No. 341191 GLP: yes unpublished	Y	

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
IIA, 2.11.2/01	van Rijsbergen, L.M.	2002	Determination of the Relative Self-Ignition Temperature of IKF-1216 TGAI. Notox B.V., Report No. 341202 GLP: yes unpublished	Y	ISK
IIA, 2.13/02	Angly H.	2005	Determination of the explosive properties of Fluazinam TGAI (IKF-1216) Institute of Safety and Security, report no. 2005.4004.EXP, May 3, 2005 GLP, unpublished	Y	ISK
IIA,2.14/01	van Rijsbergen, L.M.	2002	Determination of the Surface Tension of an Aqueous Solution of IKF-1216 TGAI. Notox B.V., Report No. 341213 GLP: yes unpublished	Y	ISK
IIA, 2.15/02	van der Baan-Treur J.	2005	Statement on the oxidizing properties of Fluazinam Notox, report no. 435072, June 15, 2005 Non-GLP, unpublished	Y	ISK
Annex III					
IIIA, 2.1/01	van Rijsbergen, L. M.	2002	Determination of the Appearance of IKF-1216 500SC. NOTOX, Report No. 341224 GLP: yes unpublished	Y	ISK
IIIA, 2.2.1/01	Widmer H.	2005	Expert statement on the explosive properties of Fluazinam 500 SC (IBE 3876) RCC Ltd, report no. A22904, July 14, 2005 Not GLP, unpublished	Y	ISK
IIIA, 2.2.2/01	De Smet B.	2005	Expert statement on the oxidizing properties of IKF-1216 500 SC under consideration of the formulants ISK Biosciences Europe SA, report no. IBE1216-PC0507-01, July 07, 2005 Not GLP, unpublished	Y	ISK
IIIA, 2.3/01	van Rijsbergen, L. M.	2002	Determination of the Auto-Ignition Temperature (Liquids) of IKF-1216 500SC. NOTOX, Report No.341235 GLP: yes unpublished	Y	ISK
IIIA, 2.4.2/01	Bird, N.R.	1993	Fluazinam: Determination of the Accelerated Storage Stability and Physico-Chemical Characteristics of a 500 g/L Suspension Concentrate Formulation. ICI Agrochemicals, Report No. RY0013B GLP: yes unpublished	N	ISK
IIIA, 2.4.2/02	Stanley,	1992	Fluazinam: Physico-Chemical Properties of	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
	R.D., Johnson, P.S.		a 500 g/L Suspension Concentrate. Imperial Chemical Industries PLC, Group Environmental Laboratory, Report No. BL4535/B GLP: yes unpublished		
IIIA, 2.5.2/01 = IIIA, 2.4.2/02	Stanley, R.D., Johnson, P.S.	1992	Fluazinam: Physico-Chemical Properties of a 500 g/L Suspension Concentrate. Imperial Chemical Industries PLC, Group Environmental Laboratory, Report No. BL4535/B GLP: yes unpublished	N	ISK
IIIA, 2.5.3/01	van Rijsbergen, L. M.	2002	Determination of the Surface Tension of an Aqueous Solution of IKF-1216 500SC. NOTOX, Report No. 341246 GLP: yes unpublished	Y	ISK
IIIA, 2.6.1/01 = IIIA, 2.4.2/01	Bird, N.R.	1993	Fluazinam: Determination of the Accelerated Storage Stability and Physico-Chemical Characteristics of a 500 g/L Suspension Concentrate Formulation. ICI Agrochemicals, Report No. RY0013B GLP: yes unpublished	N	ISK
IIIA, 2.6.1/02 = IIIA, 2.4.2/02	Stanley, R.D., Johnson, P.S.	1992	Fluazinam: Physico-Chemical Properties of a 500 g/L Suspension Concentrate. Imperial Chemical Industries PLC, Group Environmental Laboratory, Report No. BL4535/B GLP: yes unpublished	N	ISK
IIIA,2.7.1/01	Bird, N.R., Swaine, H.	1993	Fluazinam: Determination of the Accelerated Storage Stability and Physico-Chemical Characteristics of a 500 g/L Suspension Concentrate Formulation. ICI Agrochemicals, Report No. RY0003B GLP: yes unpublished	N	ISK
IIIA,2.7.1/02 = IIIA, 2.4.2/01	Bird, N.R.	1993	Fluazinam: Determination of the Accelerated Storage Stability and Physico-Chemical Characteristics of a 500 g/L Suspension Concentrate Formulation. ICI Agrochemicals, Report No. RY0013B GLP: yes unpublished	N	ISK
IIIA, 2.7.1/03 = IIIA, 2.4.2/02	Stanley, R.D., Johnson, P.S.	1992	Fluazinam: Physico-Chemical Properties of a 500 g/L Suspension Concentrate. Imperial Chemical Industries PLC, Group Environmental Laboratory,Report No. BL4535/B	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
			GLP: yes unpublished		
IIIA, 2.7.2/01	Schiffers, B.	2002	IKF-1216 500SC: Storage Stability at Low Temperature. Chimie analytique et Phytopharmacie, Cellule-Formulation, Faculte Universitaire des Sciences Agronomiques, Report No. 7-FLUAZINA 02/01 GLP: yes unpublished	Y	ISK
IIIA, 2.7.3/01	Bird, N.R., Swaine, H.	1995	Fluazinam: Determination of the Long-Term Storage Stability of a 500 g/L Suspension Concentrate Formulation in Sales Packs. ICI Agrochemicals, Report No. RY0072B GLP: yes unpublished	N	ISK
IIIA, 2.8.3/01	van Rijsbergen, L. M.	2002	Spontaneity of Dispersion of IKF-1216 500SC in Aqueous Suspension. NOTOX, Report No. 341257 GLP: yes unpublished	Y	ISK
IIIA, 2.8.8.2/01 = IIIA, 2.4.2/01	Bird, N.R.	1993	Fluazinam: Determination of the Accelerated Storage Stability and Physico-Chemical Characteristics of a 500 g/L Suspension Concentrate Formulation. Ishihara Sangyo Kaisha, Ltd., Report No. RY0013B GLP: yes unpublished	N	ISK
IIIA, 2.9.1/01	Fletcher, N.	1992	Shirlan 50SC (Fluazinam 500SC): Compatibility Testing. Evaluation of the Physical Compatibility of Tank Mixture ICI Agrochemicals, Report No. RIC 1778 GLP: yes unpublished	N	ISK
IIIA, 2.9.2/01	Versmissen, C.	2005	Compatibility assurance statement on products that could be tank mixed with Fluazinam 500 SC ISK Biosciences Europe SA, report no. IBE1216-PC0705-01, July, 2005 Not GLP, unpublished	Y	ISK

Annex B

Fluazinam

B.3 Data on application and further information

B.3 Data on application and further information

B.3.1 Data on application relevant to the active substance (Annex IIA 3.1 - 3.6)

B.3.1.1 Function (Annex IIA 3.1)

Fungicide

B.3.1.2 Effects on harmful organisms (Annex IIA 3.2)

B.3.1.2.1 Nature of the effects on harmful organisms (Annex IIA 3.2.1)

Foliar (contact protective) fungicide for the control of *Phytophthora infestans* (potato late blight and tuber blight) that needs to be applied before the disease attack.

B.3.1.2.2 Translocation in plants (Annex IIA 3.2.2)

Systemic activity (by translocation from roots to leaves) is not observed.

B.3.1.3 Field of use (Annex IIA 3.3)

Agriculture

B.3.1.4 Harmful organisms controlled and crops or products protected or treated (Annex IIA 3.4)

The active ingredient acts as a fungicide with activity against fungus from the class of *Oomycetes*, especially against *Phytophthora infestans*, both potato late blight and tuber blight. It works protectively and needs to be applied before the disease attack. Depending on the disease pressure, good protection against the disease can be expected over a period of 7 to 10 days. Protection is also observed for tubers after harvest.

B.3.1.5 Mode of action (Annex IIA 3.5)

Fluazinam is a preventive contact fungicide with a multi-site mode of action. It disrupts the production of energy at several metabolic pathways within the fungal cell. It is absorbed into the fungal spores, and is rapidly conjugated with glutathione, thereby depleting glutathione from germinating fungal spores, germ tubes, appresoria and infection pegs. This disruption in energy (ATP) production in the fungus results in the inhibition of various infectious processes including sporulation, spore motility, spore germination and hyphal penetration and growth, resulting in the death of the fungus. Fluazinam is a protectant fungicide and when applied to plants, remains primarily on the plant surface, is not taken up to any extent by the plant and is not translocated within the plant like systemic fungicides.
The first application against potato late blight is to be made when warning systems forecast indicates significant disease attack situations. The product will be applied at a maximum individual application rate per spray of 200 g a.i./ha.

B.3.1.6 Details of active metabolites and degradation products (Annex IIA 3.5)

No active metabolites and degradation products

B.3.1.7 Information on possible occurrence of the development of resistance (Annex IIA 3.6)

Because of the described “multi-site” mode of action (i.e.: disruption of energy production at multiple metabolic sites within the fungal cell), fluazinam has extremely low potential for development of resistance. Use of fluazinam in alternation or in combination with systemic fungicides will help delay on the onset of the development of disease resistance to the fungicides that have a specific or single-site mode of action.

B.3.2 Data on application relevant to the plant protection product (Annex IIIA 3)

B.3.2.1 Field of use (Annex IIIA 3.1)

Agriculture (potatoes).

B.3.2.2 Effects on harmful organisms (Annex IIIA 3.2)

Foliar (contact, protective) fungicide for the control of *Phytophtora infestans* (late blight and tuber blight) that needs to be applied before the disease attack.

B.3.2.3 Details of representative uses (Annex IIIA 3.3)

Table 3.2.3-1 Summary of representative uses evaluated (Fluazinam) (Annex IIA 3.4)

Crop and/ or situation (a)	Member State or Country	Product name	F G or I (b)	Pests or Group of pests controlled (c)	Preparation		Application				Application rate per treatment			PHI (days) (m)	Remarks:
					Type (d-f)	Conc. of as (i)	method kind (f-h)	growth stage & season (j)	number min/ max (k)	interval between applications (min)	g as/hL (l) min – max	water L/ha min – max	g as/ha (l) min – max		
Potatoes	Europe	Fluazinam 500SC	F	*Phytophtora infestans* (late blight and tuber blight)	SC	500 g/L	Field boom sprayer with hydraulic boom and nozzles	first application when warning systems forecast indicates significant disease attack last treatment BBCH 95-97	10	7 to 10 day intervals depending on the disease pressure	40 100	200 500	max. 200	7	

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (*e.g.* fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds

(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

B.3.2.4 Application rate (Annex IIIA 3.4)

See Table B.3.2.3-1

B.3.2.5 Concentration of active substance in material used (Annex IIIA 3.5)

See Table B.3.2.3-1

B.3.2.6 Method of application (Annex IIIA 3.6)

Field boom sprayer with hydraulic boom and nozzles, 1 or 2 nozzles according to the size of the band to treat. The water volume varies between 200 L/ha to 500 L/ha.

B.3.2.7 Number and timing of application (Annex IIIA 3.7)

See Table B.3.2.3-1

B.3.2.8 Necessary waiting periods or other precautions to avoid phytotoxic effects on succeeding crops(Annex IIIA 3.8)

Not relevant as the product does not cause phytotoxic effects.

B.3.2.9 Proposed instructions for use (Annex IIIA 3.9)

Product labels have been submitted.

B.3.3 Summary of data on application

Fluazinam acts as a fungicide with activity against fungus from the class of *Oomycetes*, especially against *Phytophthora infestans*, both potato late blight and tuber blight. It works protectively and needs to be applied before the disease attack in a concentration of max. 200 g as/ha.

Fluazinam 500SC is registered in several European countries. A maximum number of 10 applications is recommended, the first treatment against potato late blight should take place when warning systems forecast indicates significant disease attack and the last at growth stage BBCH 95-97 using field boom sprayers with hydraulic boom and nozzles. The typical spray volumes are between 200 to 500 L/ha.

B.3.4 Further information on the active substance (Annex IIA 3.7 - 3.9)

B.3.4.1 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIA 3.7)

Reference: BIG (2003): Safety data sheet Fluazinam tech (No Document N°)
Brandweerinformatiecentrum voor Gevaarlijke Stoffen vzw (BIG)
Guideline: 2001/58/EC
GLP: no
Handling:
Read the label before use. Observe very strict hygiene standards. Avoid contact. Avoid

raising dust. Do not discharge the waste into the drain. Remove contaminated clothing immediately. Clean contaminated clothing.

Storage:

Keep in original containers, tightly closed. Keep container in a dry and dark place. Keep away from heat sources.

Transport:

Classified as 6.1 for transport. Packing group II.

Fire:

Self-contained breathing apparatus and protective clothing should be worn when fighting fire involving chemicals.

Extinguishing media:	Water spray; polyvalent foam, alcohol-resistant foam; ABC powder; carbon dioxide.
Unsuitable extinguishing media:	Solid water jet is ineffective as extinguishing medium.
Combustion gases:	Combustion or thermal decomposition will evolve toxic and corrosive gases / vapours (nitrous vapours, hydrogen chloride, hydrofluoric acid, carbon monoxide, carbon dioxide).

B.3.4.2 Procedures for destruction or decontamination (Annex IIA 3.8)

Pyrolytic behaviour controlled incineration: Consideration of the content of halogens is not relevant (content is less than 60%).

Stability and reactivity: Upon heating/burning, there could be a release of toxic and corrosive vapors, e.g.: nitrous vapors, sulphur oxides, hydrofluoric acid, carbon monoxide and carbon dioxide. Stable under normal conditions.

Instructions for safe disposal: Remove to an incinerator for chlorinated waste materials.

B.3.4.3 Emergency measures in case of accident (Annex IIA 3.9)

First aid measures:

Eye contact:	Consult a doctor/medical service if irritation persists Rinse immediately with plenty of water Do not apply neutralizing agents
Skin contact:	Consult a doctor/medical service if irritation persists Wash immediately with lots of water Soap may be used Remove clothing before washing
After inhalation:	Remove the victim into fresh air Unconscious: maintain adequate airway and respiration Immediately consult a doctor/medical service
After ingestion:	Consult a doctor/medical service if you feel unwell

Never give water to an unconscious person
Victim is fully conscious: immediately induce vomiting
Give nothing or little to drink

Decontamination of water: Absorb on active carbon.

B.3.5 Further information on the plant protection product Fluazinam 500SC (Annex IIIA 4)

B.3.5.1 Packaging (type, materials, size, etc.), compatibility of the preparation with packaging materials (Annex IIIA 4.1)

B.3.5.1.1 Description and specification of packaging and materials used in packaging

One (1) litre:
Polyethylene terephthalate (PET) container
Size of opening: 45 mm
Types of closure and sealing: induction heat sealing + tamper-evident + HDPE screw cap

Five (5) litre:
High density polyethylene (HDPE) container with screw cap
Size of opening: 63 mm
Types of closure and sealing: induction heat sealing + tamper-evident + HDPE screw cap

The suitable stacking test was done with the combined packaging currently used for the manufacture (i.e.: 4 X 5L and 12 X 1L). The applied load during 24 hours was 213 and 190 kg respectively for the 4 X 5 L and 12 X 1 L combined packaging. No leaks on inner packaging and deterioration of outer packaging was observed compromising the safety of the transport.
In addition to the stacking test, a drop test was done with the same combined packaging from a height of 1.2 m at different drop positions. Again, no leaks on inner packaging and deterioration of outer packaging was observed compromising the safety of the transport.

B.3.5.1.2 Resistance of packaging material to its contents

Storage data at accelerated temperature demonstrate resistance and compatibility of the formulation with the packaging (see B.2.2.17).

B.3.5.2 Procedures for cleaning equipment (Annex IIIA 4.2)

Procedure:	Two complete rinsings with tap water.
Effectiveness of cleaning procedure:	Practical experience over ten years of product use have shown that this procedure is sufficient.

B.3.5.3 Re-entry periods, necessary waiting periods or other precautions to protect man,

livestock and the environment(Annex IIIA 4.3)

Preharvest interval (in days) for each relevant crop:

Pre-harvest interval is 7 days for potatoes

Re-entry period (in days) for livestock, to areas to be grazed:

Livestock are not expected to enter treated fields under normal circumstances

Re-entry period (in hours or days) for man to crops, buildings or spaces treated:

The exposure of workers re-entering treated fields was based on levels of dislodgeable foliar residues present at one hour after the final application. When the daily systemic exposure is compared with the AOEL, it is 54%, therefore the conditions of usage of fluazinam as proposed, pose no risk for re-entry one hour after treatment.

Withholding period (in days) for animal feedingstuffs:

Livestock are not fed with treated crop.

Waiting period (in days) between application and handling treated products:

Not relevant. A withholding period of one (1) day is sufficient.

Waiting period (in days) between last application and sowing or planting of succeeding crops:

Waiting period is not required as neither Fluazinam nor relevant fluazinam related metabolites are found in the succeeding crop study.

B.3.5.4 Recommended methods and precautions concerning handling, storage, transport or fire (Annex IIIA 4.4)

Handling and storage:

Information on safe handling:

Observe very strict hygiene – avoid contact
Do not discharge the waste into the drain
Remove contaminated clothing immediately
Clean contaminated clothing

Protective clothing and equipment proposed:

Measure the concentration in the air regularly
Work under local exhaust / ventilation
Eye protection : Face shield
Skin protection: Gloves Protective clothing
Respiratory protection
(high vapour concentration): Gas mask

Information on storage:

Keep only in original containers
Keep away from heat sources, ignition sources
Keep container tightly closed
Provide for a tub to collect spills

Transport:

Substance identification number (UN Number.):	03082
Transport by road / rail (ADR/RID):	class 9
Danger code:	90
Maritime transport (IMDG code):	class 9 p
Transport by inland ways (ADNR):	class 9
EMS:	-
MFAG:	-
Inland navigation (ADNR):	class 9
Air freight (ICAO):	class 9

Fire:

Extinguishing media:

All extinguishing media are allowed including water spray, polyvalent foam, BC powder and carbon dioxide.

Take account of toxic firefighting water.

Dilute toxic gases with water spray.

Heat/fire exposure:

Compressed air/oxygen apparatus

Gas-tight suit

Protective clothing and equipment proposed:

Fluazinam 500SC is irritant to eyes and sensitizer to skin.

Wear suitable protective clothing (overalls) and suitable protective gloves when handling the concentrate. If the ventilation is insufficient wear respiratory protection.

Wash all protective clothing thoroughly after use, especially the insides of gloves.

Handle with care and mix only in a close container.

Avoid all contact with skin.

Wash hand and exposed skin before meals and after work.

Eye protection: face shield

Suitable materials for gloves and protective clothing are rubber, plastics, PVC

Suitability and effectiveness of protective clothing and equipment:

The protective clothing and equipment proposed are standard items for the protection of workers handling agricultural products. The safe use of fluazinam over the past ten years demonstrates the suitability and effectiveness of the clothing and equipment under actual use conditions.

B.3.5.5 Emergency measures in case of an accident (Annex IIIA 4.5)

Prevent soil and water pollution. Do not discharge into the sewer. Plug the leak, cut off the supply. Dam up the liquid spill. Absorb the liquid spill into an absorbent agent and scoop the absorbed substances into sealable containers. Clean contaminated surfaces with an excess of water. Carefully collect the spill and any residue. Wash clothing and equipment after handling.

Protection of emergency workers and bystanders:

Use the protective equipment proposed above.

Information on combustion products likely to be generated in the event of fire:

In the event of fire, the release of harmful vapours and gases must be anticipated.

First Aid:

Upon inhalation:	Consult a doctor / medical service
	Remove the victim into fresh air
Following skin contact:	Consult a doctor / medical service
	Wash immediately with lots of water / soap (15 min.)
	Remove clothing before washing
Following eye contact:	Consult a doctor / medical service
	Rinse immediately with plenty of water for 15 min.
Upon swallowing:	Consult a doctor / medical service
	Never give water to an unconscious person
	Do not induce vomiting

B.3.5.6 Procedures for the destruction or decontamination of the plant protection product and its packaging (Annex IIIA 4.6)

Neutralisation procedures:

Absorb the liquid spill into an absorbant agent and scoop. (See MSDS)

Detailed instructions for safe disposal of the plant protection product and its packaging:

Remove to an incinerator for chlorinated waste materials

Pyrolitic behaviour of the active substance under controlled conditions at 800 °C and the content of polyhalogenated dibenzo-p-dioxins in the products of pyrolysis:

Not applicable. Consideration of the content of halogens is not relevant (content is less than 60%).

Methods other than controlled incineration for disposal of the plant protection product, contaminated packaging and contaminated materials:

Remove to an authorized waste treatment plant.

B.3.6 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					
IIA, 3.7	BIG	2003	Safety data sheet Fluazinam tech. Brandweerinformatiecentrum voor Gevaarlijke Stoffen vzw (BIG)	N	ISK
Annex III					
IIIA, 4.1.2/01	Fragu, E.	2002	Mechanical Tests on a Type of Packaging Bureau de Vérifications Techniques, Report No. 5409 Not GLP, unpublished	Y	ISK
IIIA, 4.1.2/02	Fragu, E.	2002	Mechanical Tests on a Type of Packaging Bureau de Vérifications Techniques, Report No. 5401 Not GLP, unpublished	Y	ISK

Annex B

Fluazinam

B.4 Proposal for classification and labelling

B.4 Proposals for classification and labelling

B.4.1 Proposals for the classification and labelling of the active substance (Annex IIA 10)

On the basis of the available data the following classification and labelling is proposed according to Directive 67/548/EEC in combination with Directive 93/21/EEC:

Hazard symbols:		Skull over crossed bones Dead fish, dead tree
Indication of danger:	T Xi N	Toxic Irritant Dangerous for the environment
Risk phrases:	R 23	Toxic by inhalation
	R 41	Risk of serious damage to eyes
	R 43	May cause sensitisation by skin contact
	R 50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
	R 63	Possible risk of harm to the unborn child
Safety phrases:	S 1	Keep locked up
	S 2	Keep out of the reach of children
	S 13	Keep away from food, drink and animal feeding stuffs
	S 20/21	When using do not eat, drink or smoke
	S 24/25	Avoid contact with skin and eyes
	S 26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
	S 27/28	After contact with skin, take off immediately all contaminated clothing and wash immediately with plenty of water
	S 36/37/39	Wear suitable protective clothing, gloves and eye/face protection
	S 38	In case of insufficient ventilation, wear suitable respiratory equipment
	S 45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
	S 56	Dispose of this material and its container to hazardous or special waste collection point.
	S 57	Use appropriate container to avoid environmental

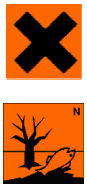
		contamination.
	S 60	This material and its container must be disposed of as hazardous waste.
	S 61	Avoid release to the environment. Refer to special instructions/safety data sheets.
	S 63	In case of accident by inhalation: remove casualty to fresh air and keep at rest

Justification for the proposal:

T	Follows from R 23
Xi	Follows from R 41 and R 43
N	Follows from R 50/53
R 23	Acute inhalative LC_{50} in rats (4h, whole body exposure) 0.46 mg/l (Tobeta Y., 1988)
R 41	Corneal, iridal and conjunctival effects which persisted partly through day 21 of the study (Shults S. K., 1992)
R 43	Sensitization in the Magnusson and Kligman maximization study and in the method of Buehler (Cummins H. A.., 1984; Pritchard V. A., 1986)
R 50/53	Follows from the toxicity to fish (*Oncorhynchus mykiss* LC_{50} = 0.036 mg/L, Gelin & Laveglia 1992), the Log_{POW} of active substance is 4.03 (Sanders, 1992) and the active substance is not ready biodegradable (Grützner, 2000).
R 63	Gross morphological fetal abnormalities (cleft palate, diaphragmatic hernia) at maternal toxic doses (Willoughby C. R., 1985)
S 1, S 2, S 13, S 20/21, S 36/37/39, S 45	Proposed because fluazinam is toxic by inhalation, has risk of serious damage to eyes, may cause sensitization by skin contact and is teratogenic
S 24/25	Proposed because fluazinam has risk of serious damage to eyes and may cause sensitization by skin contact
S 26	Proposed because fluazinam has risk of serious damage to eyes
S 27/28	Proposed because fluazinam may cause sensitization by skin contact
S 38, S 63	Proposed because fluazinam is toxic by inhalation
S 56, S 57, S 60, S 61	Proposed because the toxicity of fluazinam to aquatic organisms and potential long term adverse effects to the aquatic environment

B.4.2 Proposals for the classification and labelling of preparations (Annex IIIA 12.3 and 12.4)

On the basis of the available data the following classification and labelling is proposed according to Directive 67/548/EEC in combination with Directive 93/21/EEC:

Hazard symbols:		Dead fish, dead tree
Indication of danger:	Xn Xi N	Harmful Irritant Dangerous for the environment
Risk phrases:	R 43	May cause sensitisation by skin contact
	R 50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
	R 63	Possible risk of harm to the unborn child
Safety phrases:	S 1	Keep locked up
	S 2	Keep out of the reach of children
	S 13	Keep away from food, drink and animal feeding stuffs
	S 20/21	When using do not eat, drink or smoke
	S 24	Avoid contact with skin
	S 27/28	After contact with skin, take off immediately all contaminated clothing and wash immediately with plenty of water
	S 36/37/39	Wear suitable protective clothing, gloves and eye/face protection
	S 45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
	S 56	Dispose of this material and its container to hazardous or special waste collection point.
	S 57	Use appropriate container to avoid environmental contamination.
	S 60	This material and its container must be disposed of as hazardous waste.
	S 61	Avoid release to the environment. Refer to special instructions/safety data sheets.

Justification for the proposal:

Xn	Follows from R 63
Xi	Follows from R 43

N	Follows from R 50/53
R 43	Sensitization in the Magnusson and Kligman maximization study and in the method of Buehler (Smith K. D., 1992; Lees D., 1991)
R 50/53	Follows from the toxicity to fish (*Oncorhynchus mykiss* LC_{50} = 0.0611 mg/L, Sankey et al 1991), the Log_{POW} of active substance is 4.03 (Sanders, 1992) and the active substance is not ready biodegradable (Grützner, 2000).
R 63	Gross morphological fetal abnormalities (cleft palate, diaphragmatic hernia) at maternal toxic doses (Willoughby C. R., 1985)
S 1, S 2, S 13, S 20/21, S 24, S 27/28, S 36/37/39, S 45	Proposed because fluazinam 500 SC may cause sensitization by skin contact and is teratogenic
S 56, S 57, S 60, S 61	Proposed because the toxicity of fluazinam 500 SC to aquatic organisms and potential long term adverse effects to the aquatic environment

B.4.3 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					
IIA, 5.2.6	Cummins H. A.	1984	Delayed contact hypersensitivity study in guinea-pigs [redacted] Report No.: 84/ISK054/686 GLP: yes Unpublished	Y	ISK
AII 8.2.1	Gelin, M.D, Laveglia, J.	1992	Technical Fluazinam (IKF-1216) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Generated by: [redacted] Report No: 5099-91-0422-TX-002 GLP / GEP: yes unpublished	N	ISK
AII, 7.2.1.3.1	Grützner, I.	2000	Ready Biodegradability of Fluazinam in a Manometric Respirometry Test. RCC Ltd Report No. 774898 GLP, unpublished	Y	ISK
IIA, 5.2.6	Pritchard V.	1986	Skin sensitisation to the guinea-pig of both the purified and technical material [redacted] Report No.: CTL/P/1493 GLP: yes Unpublished	Y	ISK
AII, 2.8	Sanders, J.M.	1992	Fluazinam (IKF-1216) (ASC-66825) - Octanol/Water Partition Coefficient. Ricerca, Inc. Report No. 4039-91-0386-AS-001 GLP: yes unpublished	N	ISK
IIA, 5.2.5	Shults S. K.	1992	Primary eye irritation study in albino rabbits with IKF-1216 [redacted] Report No.: 5016-91-0280-TX-002 GLP: yes Unpublished	Y	ISK
IIA, 5.2.3	Tobeta Y.	1988	Acute inhalation toxicity test of fluazinam in rats [redacted] Report No.: D-1775E GLP: yes Unpublished	Y	ISK
IIA, 5.6.2	Willoughby C.	1985	B-1216: Teratology study in the rat	Y	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
	R.		Life Science Research Ltd. Report No.: 84/ISK047/606; Amended Final Report No.: 91/ISK047/0820 GLP: yes Unpublished		
Annex III Data and Information					
AII, 7.2.1.3.1	Grützner, I.	2000	Ready Biodegradability of Fluazinam in a Manometric Respirometry Test. RCC Ltd Report No. 774898 GLP, unpublished	Y	ISK
IIIA 7.1.6	Lees D., Robinson P.	1991	ICIAO192: Skin sensitisation t the guinea pig of a 500 g/l SC formulation ICI Central Toxicology Laboratory, Alderley Park, UK Report No.: CTL/P/3227 GLP: yes Unpublished	Y	ISK
AII, 2.8	Sanders, J.M.	1992	Fluazinam (IKF-1216) (ASC-66825) - Octanol/Water Partition Coefficient. Ricerca, Inc. Report No. 4039-91-0386-AS-001 GLP: yes unpublished	N	ISK
AIII, 10.2.1	Sankey, S.A., Tapp, J.F., Caunter, J.E., Penwell, A.J.	1991	Fluazinam: Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) of a 500 g/L SC Formulation. Generated by: Imperial Chemical Industries PLC, Group Environmental Laboratory Report No: BL4323/B GLP / GEP: yes unpublished	N	ISK
IIIA 7.1.6	Smith K. D.	1990	IKF-1216 50 % SC: Dermal sensitisation study in guinea pigs Life Science Research Ltd. Report No.: 90/ISK159/1205; GLP: yes Unpublished	Y	ISK
IIA, 5.6.2	Willoughby C. R.	1985	B-1216: Teratology study in the rat Life Science Research Ltd. Report No.: 84/ISK047/606; Amended Final Report No.: 91/ISK047/0820 GLP: yes Unpublished	Y	ISK

Annex B

Fluazinam

B.5 Methods of analysis

B.5 Methods of analysis

B.5.1 Analytical methods for technical active substance and formulation analysis (Annex IIA 4.1, Annex IIIA 5.1)

B.5.1.1 Methods for the analysis of pure active substance in the active substance as manufactured (Annex II 4.1.1 and 4.1.3)

Confidential information, see Volume 4, Annex C.

Applicability of existing CIPAC methods:

No CIPAC method is issued for analysis of the active substance in the TGAI at the time of evaluation.

B.5.1.2 Determination of isomers, impurities and additives in the active substance as manufactured (IIA 4.1.2)

Confidential information, see Volume 4, Annex C.

B.5.1.3 Plant protection product (IIIA 5.1)

Reference: Krainz, A. (2001): Validation of an Analytical Method for the Determination of Fluazinam (Active Ingredient) in IKF-1216 500SC (Report No 838923)

Guideline: Guidance for generating and reporting methods of analysis in support of pre- and post-registration data requirements for Annex II (part A, Section 4) and Annex III (part A, Section 5) of Directive 91/414. “Working document”. SANCO/3030/99 rev.4 11/07/00.

GLP: yes

Validity: The method is considered acceptable.

Principle of the method:

Internal standard solution (dimethylphthalate in acetonitrile) were added to IKF-1216 500SC formulation blank and filled up with acetonitrile. This results in an amount of 0.1 µg/mL IKF-1216 500SC formulation blank in the final solution. 3 different amounts of IKF-1216 (fluazinam) analytical standard (99.8%) were added to this solution to obtain approximately 100 µg/mL in the final solution for the determination of accuracy and precision.

For the determination of the retention time and linearity standard solutions containing 80.5, 99.9 and 121.6 µg/mL fluazinam were prepared.

The determination is performed by isocratic reversed stationary phase (Hypersil C18, 5µm, 250 mm x 4.0 mm) HPLC. The active substance is detected in the eluate [water (0.1% Na_2HPO_4 adjusted to pH 3.0 with citric acid (0.2 mol/L))/ acetonitrile (30/ 70 v/v)] by UV absorption at 255 nm.

Findings:

Specificity/ interferences	Linearity	Accuracy n = 3		Repeatability n = 5
		fortification level (µg/mL)	mean recovery (%)	(% RSD)
no interferences, demonstrated by chromatograms	r^2 = 0.9997 3 levels (duplicate at one level) 80.5 - 121.6 µg/mL	~100	99.3 % RSD = 0.2	0.3

For demonstrating linearity, only at one level (of 3) a duplicate determination is performed (demonstrated in amount/area counts and the graph of the linearity curve). However, in the method description duplicate injections of the calibration standards are mentioned.

Conclusion:

The method is sufficiently validated. Additional data to demonstrate linearity according to SANCO 825/00 should be documented.

Reference: Tandy, M.J. (1996): The Determination of Fluazinam in Technical and Formulated Materials by High Performance Liquid Chromatography. Zeneca Agrochemicals, unpublished, (Report No. PAM 600)

Guideline: None specified

GLP: no

Validity: The method is considered not acceptable.

Principle of the method:

Samples containing approximately 0.1 g fluazinam are dissolved in 100 mL acetonitrile and diluted (if required under addition of an internal standard) 1:10. The determination is performed by isocratic reversed phase HPLC [Spherisorb S5 ODS2, 25 cm x 0.5 cm (i.d.)]. The active substance is detected in the eluate (acetonitrile / water / glacial acetic acid 70/29.5/0.5 v/v) by UV absorption at 255 nm.

Conclusion:

Validation data are insufficient. (One point calibration using peak height comparison, no individual data for the determination of the precision and accuracy; only conclusions).

Applicability of existing CIPAC methods:

No CIPAC method is issued for analysis of the active substance in the formulation at the time of evaluation.

B.5.1.3.1 Determination of isomers, impurities and additives in the formulation (IIIA 5.1.2)

The analytical method for impurity 5, which is considered as non-relevant by the notifier in contrast to RMS, is added to Volume 4, Annex C till final clarification.
The analytical method for impurity 6, which is also considered as relevant by RMS, is missing.

B.5.2 Analytical methods (residue) for plants, plant products, foodstuffs of plant and animal origin, feedingstuffs (Annex IIA 4.2.1, Annex IIIA 5.2.1)

B.5.2.1 Analytical methods (residue) for plants and plant products

Residues of fluazinam in grapes, wine and potatoes

Reference: Wais, A. (2002): Validation of the Residue Analytical Method for Fluazinam in Potato (RAC Tubers), Grapes and Wine, RCC Ltd., unpublished, (Report No. 845972)
Guideline: Residue Analytical Method, Commission Directive 96/46/EC, July 16, 1996 and European Commission, Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 6, Jun. 20, 2002
GLP: yes
Reference: Wais, A. (2005): 1st Amendment to compilation report; Validation of the Residue Analytical Method for Fluazinam in Potato (RAC Tubers), Grapes and Wine, RCC Ltd., unpublished, (Report No. 845972)
Guideline: --
GLP: no
Validity: The method is considered acceptable but a confirmatory technique is required to demonstrate specificity.
Principle of the method:
Acetic acid (2 mL) and 100 mL of methanol were added to the fortified samples.
Potato and grape samples:
The suspension was treated for 1-2 min with an Ultra Turrax, which was then rinsed with 10-20 ml of methanol. The suspension was filtered over Celite. The extraction flask and filtercake were rinse three times with 20 mL each of methanol. The combined filtrates were transferred to a 200-mL measuring flask and made up to volume with methanol.
Wine:
The sample was shaken for 15 min. The solutions were transferred into a 200 mL measuring flask using methanol and made to volume with methanol.
The following steps are equal for all commodities:
An aliquot of 50 mL is transferred to a separatory funnel and 150 mL of 0.2 M HCl is added. Liquid-liquid separation is carried out using 50 mL of hexane followed by another 30 mL of

hexane by shaking for 1-2 min, each. The combined hexane phases were re-extracted using two time 80 mL of 0.5 M NaOH by shaking 1-2 min, each. The hexane phase is discarded. The pH of the combined NaOH solutions is adjusted to 1 by addition of about 10 mL HCl. Liquid-liquid separation is carried using 50 mL of hexane followed by another 30 mL of hexane by shaking for 1-2 min, each. The aqueous phase is discarded. The hexane layers are filtered separately over 5-10g of sodium sulphate. The sodium sulphate is rinsed using 20-30 mL of hexane. Hexane phases are evaporated to dryness using a rotary evaporator at about 40 ºC.

After a clean-up step using Florisil columns the concentration of Fluazinam is determined by GC-ECD. Columns DB 5 (30 m x 0.32 mm 0.25 µm) or DB 1 (10 m x 0.32 mm 0.25 µm) useable for potatoes and DB 5 (30 m x 0.32 mm 0.25 µm) or DB 1 (20 m x 0.32 mm 0.25 µm) for grapes and wine.

Findings:

Examples of calibration curves are provided and show linearity between 0.005 and 0.5 µg/mL (r^2 = 0.996 for potatoes and r^2 = 0.9987 for grapes and wine, respectively). The LOQ is set at 0.01mg/kg for each matrix. No interferences above 30% of LOQ were detected at the retention time of fluazinam.

Validation data are summarized in Table B.5.2.

Conclusion:

The method is suitable as enforcement method for potatoes, grape and wine.

No sufficient confirmatory method has been submitted and is therefore required.

The 1st amendment corrects the typing errors within the original study, where in some cases µg/kg or µg/L has been written wrongly instead of mg/kg and mg/L.

Independent laboratory validation for potato:

Reference: Brachet, A., Nésa, C. (2002): Independent Laboratory Validation (ILV) of the Analytical method RCC-845972 for the Determination of Residues of Fluazinam (IKF-1216) in Potato. Battelle, unpublished, (Report No. A22-02-04)

Guideline: Residue Analytical Method, Commission Directive 96/46/EC, July 16, 1996 and European Commission, Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 6, Jun. 20, 2002

GLP: yes

Validity: The method is considered acceptable but due to an insufficient linearity range additional clarification is required.

Principle of the method:

Minor modifications to the validated method (report no. 845972) are made to simplify the analytical procedure and to improve its ruggedness:

A lower amount of sodium sulphate in the final hexane layer up was demonstrated to be sufficient. Before the Florisil clean up, the dried residue was dissolved in hexane instead of a mixture hexane/ethyl acetate (95/5). The Florisil batch was systematically deactivated before

use to ensure a better control of the Florisil SPE clean up and to render the procedure independent of Florisil batch to batch activity variations. An aliquot of the filtrate was pipetted onto the Florisil column instead of the entire filtrate. A higher amount of ethyl acetate (20% instead of 5%) in the mixture hexane/ethyl acetate was used for the elution of Fluazinam. Therefore a lower amount elution solvent was required to perform the complete Fluazinam elution. The final residue was simply dissolved in hexane and was not concentrated.
The GC column used was an Optima-5, i.e. a less polar column than the requested column (DB-608). The Optima-5 column used was demonstrated to be also appropriate for the Fluazinam determination.

The GC-ECD method [column: Optima-5 (Macherey-Nagel), 25 m × 0.25 mm I.D., 0.50 µm film] is confirmed additionally by GC/MSD [DB-17 (J&W), 30 m × 0.32 mm I.D., 0.25 µm film] in TIC mode (50 – 500 m/z) to confirm the peak identity of Fluazinam.

Findings:

No interference peak above 30% of the limit of quantification was detected at the retention time for Fluazinam in any control sample.
The calibration curve is plotted in the range of 0.005 to 0.1 µg/mL (correlation coeff.: 0.9954 for GC-ECD and r^2 = 0.991 for GC/MSD). It does not cover the demanded +20% of the upper limit of the fortification level, unless a dilution step occurred, which is not reported in the study (the range should be at least up to 0.12 µg/mL). Clarification is required.
Validation data are summarized in Table B.5.2.

Conclusion:

The linearity range is not in line with guidance document SANCO 825/00.

Residues of fluazinam in coffee, grapes (fruit, must and wine), onion, potato and wine:

Reference: Ryan, J., Sapiets, A. (1991): The Determination of Residues of Fluazinam in Crops (A gas-liquid chromatography method) ICI Agrochemicals (Report No ARAM 87/1)

Guideline: -

GLP: no

Validity: The method is considered acceptable for the matrices potato, grape and wine but not for onion and coffee. A confirmatory technique is required.

Principle of the method:

The analytical method involves initial extraction of fluazinam from homogenized (20 g) crop samples (fortified with a known amount of fluazinam) using methanol. After filtration under vacuum, an aliquot is diluted with pH 7 buffer and partitioned twice (50 mL each) with dichloromethane. The organic phase is evaporated to dryness and dissolved in hexane. Only coffee samples pass an additional liquid-liquid partition chromatographic clean up step. Afterwards samples are cleaned up on a silica cartridge. Extract eluant is evaporated to dryness and residue is dissolved in acetone. Fluazinam is quantified by GLC-ECD (column:

Restek RT-5 capillary column 15 m × 0.32 mm I.D., 0.25 or 0.5 µm film thickness) which results in different retention times [9.8 minutes (0.25 µm film) or 21 minutes (0.5 µm film)].
For few samples e.g. coffee seeds, which may cause interfering peaks at the RT of fluazinam an alternative HPLC method is recommended increasing the concentration in the final solution to 2 – 5 g/mL to achieve a LOQ of 0.1 mg/kg. Acetonitrile / water + 0.4% glacial acetic acid 60/40 v/v as mobile phase and a Spherisorb 5 ODS2, 25 cm x 0.5 cm (i.d.) column is used for the determination at 258 nm.

Findings:

The submitted detector calibration graph is linear (correlation coefficient = 0.998) between approx. 0.01 to 0.5 µg/mL, but the equation of the graph is missing.
According to guidance document SANCO 825/00 the LOQ is set at the lowest fortification level for sufficiently validated matrices and is therefore for grape 0.1, for potato 0.02 and for wine 0.05 mg/kg.
Validation data are summarized in Table B.5.2.

Conclusion:

The method is successfully validated in accordance to guidance doc. SANCO 825/00 excluding the commodities onion and coffee due to the numbers of samples do not meet the requirements of SANCO 825/00.
A HPLC method is reported, but no validation data are submitted, therefore a confirmatory method validation is required.

Residues of fluazinam and its metabolites HYPA and MAPA in grapes, wine and potatoes:

Reference: Schulz H. (1992): Determination of the Residues of IKF-1216 (Fluazinam) and Its Metabolites in Grapes, Wine and Potatoes; Amendments 7, 8, 9 and 10, RCC Umweltchemie AG, unpublished, (Report No. 284670)

Guideline: -

GLP: The study was performed in compliance with OECD Principles of GLP (1981) and GLP in Switzerland, Procedures and Principles (1986) except that the field part was not performed in compliance with GLPs.

Validity: The method is considered not acceptable as the sample numbers are not according to guidance document SANCO 825/00, but at the moment the metabolites HYPA and AMPA are not included in the residue definition and therefore no further action is required.

Principle of the method:

Fluazinam Analysis – Potatoes, Grapes and Wine

A 20 g sample (either potato, grape or wine) was fortified with fluazinam at either 0.01, 0.02 or 0.1 mg/kg fortification level and extracted using a methanol:acetic acid extraction solvent (100:2, v/v). Following homogenization, the solution was suction-filtered on Celite and the filter cake washed with methanol. The final volume of filtrate was adjusted to 200 mL using

methanol.

A 50 mL aliquot was acidified with 150 mL of 0.2 N HCl and extracted twice (50 then 30 mL) with n-hexane. The organic phases were combined and extracted twice with 80 mL portions of 0.5 N NaOH. The aqueous phases were combined, adjusted to pH 1 with concentrated HCl and extracted twice (50 then 30 mL) with n-hexane. The organic phases were combined, dried by filtration on anhydrous sodium sulfate, and collected in a round-bottom flask. The n-hexane was rotary-evaporated to dryness under reduced pressure.

The residues derived from the liquid-liquid partitions were dissolved in 5 mL of n-hexane:diethyl ether (9:1), and cleaned up using a Florisil column (fluazinam was collected in the 8:2 n-hexane:diethyl ether fraction). The eluate containing fluazinam was collected, concentrated to near dryness and taken to volume in 9:1 dodecane:acetone for analysis by gas chromatography with electron capture detector (GC-ECD) using a 10m x 0.35 mm DB-1 column.

MAPA Analysis - Potatoes, Grapes and Wine

20 gram samples of potato were fortified with MAPA at 0.01, 0.02 or 0.1 mg/kg fortification levels. In a like manner, either grapes or wine (20 g samples) were fortified with MAPA at 0.01, 0.02, 0.2 or 0.1 mg/kg. The samples were extracted using a methanol:acetic acid extraction solvent (100:2, v/v). Following homogenization, the solution was suction-filtered on Celite and the filter cake washed with methanol. The final volume of filtrate was adjusted to 200 mL using methanol.

A 50 mL aliquot was acidified with 150 mL of 0.2 N HCl and extracted twice (50 then 30 mL) with n-hexane. The organic phases were combined and successively washed with 80 mL of 0.5 N NaOH (twice), 80 mL of 0.2N HCl (once), and 80 mL of 2% sodium sulfate solution (twice). The organic phase was dried by filtration on anhydrous sodium sulfate, and collected in a round-bottom flask. The n-hexane was rotary-evaporated to dryness under reduced pressure.

The residues derived from the liquid-liquid partitions were dissolved in 5 mL of n-hexane:diethyl ether (9:1), and cleaned up using a Florisil column. The eluate containing MAPA was collected (7:3 n-hexane:diethyl ether), concentrated to near dryness and taken to volume in 9:1 dodecane:acetone (v/v) for analysis by GC-ECD using a DB-210 20m x 0.32 mm column.

HYPA Analysis - Potatoes, Grapes and Wine

50 gram samples of potato were fortified with HYPA at 0.1 mg/kg fortification level. In a like manner, either grapes or wine (50 g samples) were fortified with HYPA at 0.01, 0.02 or 0.05 mg/kg. The samples were extracted using a methanol:acetic acid extraction solvent (100:2, v/v). Following homogenization, the solution was suction-filtered on Celite and the filter cake washed with methanol. The final volume of filtrate was adjusted to 300 mL using methanol.

A 150 mL aliquot was acidified with 150 mL of 0.2 N HCl and extracted twice (50 then 30 mL) with dichloromethane. The dichloromethane phases were combined and extracted with 100 mL of 0.2 N NaOH. Any emulsion was treated by centrifugation of the solution for 10

minutes at about 4000 rpm. After discarding the dichloromethane phase, the aqueous phase was adjusted to pH 1 with concentrated HCl and extracted twice (50 then 30 mL) with dichloromethane. The dichloromethane was rotary-evaporated to near dryness under reduced pressure and residual solvent was removed in a stream of nitrogen. The residue was dissolved in 2 mL of 5% phosphoric acid. The samples were then methylated with a solution of freshly generated diazomethane in diethyl ether (15 mL). The diethyl ether was removed in a stream of nitrogen, the residue was dissolved in 80 mL of 2% sodium sulfate solution and extracted with 50 mL of n-hexane. The aqueous phase was discarded and the n-hexane was re-extracted with 80 mL of 2% sodium sulfate solution. The n-hexane was dried over anhydrous sodium sulfate, decanted and extracted with 50 mL of acetonitrile. The acetonitrile was concentrated to near dryness under reduced pressure and residual solvent was removed in a stream of nitrogen. The dry residue was dissolved in 5.0 mL of 9:1 dodecane:acetone and analyzed by gas chromatography with electron capture detector (GC-ECD) using a 30m x 0.32 mm DB-1 column.

Findings:

Linear between 0.01 and 0.5 µg/mL (R^2 = 0.993). All fortification levels are insufficiently validated.

Validation data are summarized in Table B.5.2.

Conclusion:

The method is not sufficiently validated for the determination of residues of fluazinam, AMPA and HYPA.

In the case of including the metabolites AMPA and HYPA to the residue definition, a new validation is required.

GLP compliance is not necessary for analytical residue methods.

Applicability of a multi residue method:

Reference: Specht, W. (1992)

The applicability (study plan) of a multiresidue method (e.g. according to DFG 19) is reported in German for residues of fluazinam in potatoes. It should be submitted in English and validated according to guidance document SANCO 825/00.

5.2.2 Analytical methods (residue) for food of animal origin

No residue definition is established for food of animal origin, therefore no analytical method is required.

Table B.5.2 Validation data for analytical methods for the determination of residues of the active substance in food of plant and animal origin

References	Detection method	Determined analyte	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	n
Wais, A. (2002), (845972) (enforcement)	GC-ECD	Fluazinam	Potato	0.01	0.01 0.10	96.1 92.8	13.3 13.1	5 5
			Grape	0.01	0.01 0.10	102.1 96.2	9.6 19.0	5 6
			Wine	0.01	0.01 0.10	91.8 95.1	13.9 15.4	3 3
Brachet, A., Nésa, C. (2002), (A22-02-04) (ILV)	GC-ECD	Fluazinam	Potato	0.01	0.01 0.10	87.6 112.0	7.1 19.0	5 5
	GC-MSD			0.01	0.01 0.10	95.0 104.9*	19.7*	1 2
Ryan, J., Sapiets, A. (1991), (ARAM 87/1)	GLC-ECD	Fluazinam	Coffee	0.10	0.10	72	16	4
			Grape	0.10	0.05 [1)] 0.10 0.2 1.0	87.6* 82.9* 81.5* 94.3*	23.0* 16.5* 12.2* 5.3*	6 14 4 6
			Onion	0.05	0.05 0.10	78.5* 75*	13.5* 7.5*	2 2
			Potato	0.02	0.02 0.05 0.10 0.20	105.5* 87.4* 77* 71.7*	11.7* 16.2* 12.6* 8.8*	4 28 8 7
			Wine	0.05	0.02 0.05 0.10	77* 86.4* 87*	12.9* 17.8* 4:9*	2 10 2

References	Detection method	Determined analyte	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	n
Schulz H. (1992), (284670)	GC-ECD	Fluazinam	Potato	**	0.01 0.02 0.1	105.9 140.3 78.8		1 1 1
			Grape	**	0.01 0.02 0.1	102.4 84.6 67.5		1 1 1
			Wine	**	0.01 0.02 0.1	100.1* 71.3 96.8*	2.0* 17.6*	2 1 2
		MAPA	Potato	**	0.01 0.02 0.1	112.8 94.6 71.1		1 1 1
			Grape	**	0.01 0.02 0.1	75.3 115.8 72.1		1 1 1
			Wine	**	0.01 0.02 0.1 0.2	104.2 99.8* 98.7* 87.7	 1.7* 19.4* 	1 2 3 1
		HYPA	Potato	**	0.1	60.7*	16.4*	3
			Grape	**	0.01 0.02 0.05	22.1 41.5 88.7		1 1 1
			Wine	**	0.05	85.3*	7.4*	3

* calculated by RMS since only means and RSDs over all fortification levels are provided
** setting of a LOQ is not possible
marked grey values are not covered by the reported linearity range
[1)] Fortification level not included in the validation since the RSD is >20%

B.5.3 Analytical methods (residue) for soil, water, air

B.5.3.1 Soil (Annex IIA 4.2.2, Annex IIIA 5.2.2)

Reference: Ryan, J., Sapiets, A. (1992): The Determination of Residues of Fluazinam in Soil (A gas liquid chromatography method). ICI Agrochemicals, unpublished,
(Report No. ARAM 211)
Guideline: None specified
GLP: no

Reference: Sapiets A. (2005): Statement on specification of soil. Syngenta (no report number – statement not signatured)
Guideline: --
GLP: no
Validity: The method is considered acceptable, but a confirmatory technique is missing.
Principle of the method:
Soil is extracted by refluxing in acetonitrile. After filtration under vacuum aliquots of the extract (equivalent to 1 g of sample) are cleaned up on an activated SEP-PAK C18 cartridge [where interfering coextractives have not been removed by the SEP-PAK C18 cartridge, an alternative adsorbant (Silica BOND ELUT™) is recommended] and eluted with acetonitrile. The eluates were evaporated to dryness, dissolved in acetone and analyzed by gas-liquid chromatography (J7W DB1 megabore column 15 m x 0.53 mm internal diameter, 1.5).
Specification of soil: The notifier stated that some of the recovery data clearly derived from the soil dissipation study conducted in Germany (report RJ1368B).

Site and Site reference	Soil profile (cm)	Organic Matter %	Texture Analysis	Particle Size			pH	Cation Exchange Capacity mEq/100g dry soil
				Sand	Silt	Clay		
Varendorf/B1	0-10	1.3	Loamy sand	76	20	4	6.1	4.8
Klein Zecher/B2	0-10	1.5	Sandy loam	70	26	4	6.1	4.8
Ottersweier/E1	0-30	0.8	Clay	36	42	22	5.3	7.8
Sollern /G1	0-30	2.2	Clay loam	27	40	33	6.8	14.3

Findings:
Validation data of the method only using Silica BOND ELUT cartridges are summarised in Table B.5.3 since the data of alternately recommended SEP-PAK C18 cartridges are not validated according SANCO 825/00 (insufficient amount of samples at each fortification level). No peaks have been observed to interfere with fluazinam during the final chromatographic determination step.
The calibration curve is linear (seven levels) in the range of 0.01 to 0.5 µg/mL with a correlation coefficient of 0.997. The range covers only the fortification levels 0.05 to 0.75

mg/kg, calculated according to the provided analytical procedure. Therefore, the LOQ was set to 0.05 mg/kg.

Conclusion:

A confirmatory technique is required.

Reference: Burke, S., Sapiets, A., Gentle, W. (1993): The Determination of the Fluazinam Metabolite "HYPA" in Soil, A High Performance Liquid Chromatography Method. ICI Agrochemicals, unpublished, (Report No RAM/218/01)

Guideline: None specified

GLP: no

Reference: Sapiets A. (2005): Statement on specification of soil. Syngenta (no report number – statement not signatured)

Guideline: --

GLP: no

Validity: The method is considered acceptable, but a confirmatory technique is missing.

Principle of the method:

Soil samples were mixed acetonitrile refluxed for 3 hours. The extract was filtered under vacuum and brought to volume with acetonitrile. An aliquot of the extract (equivalent to 4 g of sample) was evaporated to dryness, dissolved in dichloromethane/hexane/glacial acetic acid (50:50:0.05) and loaded onto a Silica BOND ELUT™ cartridge and eluted with dichloromethane/hexane/glacial acetic acid (80:20:0.2). The eluate was evaporated to dryness, dissolved in acetonitrile/pH 4.5 acetate buffer (50:50) + 3% methanol and analyzed by HPLC [column: Kromasil 100-5C_{18} column 25 cm x 4.5 mm ID; mobile phase: acetonitrile/pH 4.5 acetate buffer (50:50) + 3% methanol flowing at 1 mL/min. If interfering peaks are present, up to 5% methanol can be used] with UV detection at 250 nm.

The specification of soil samples were defined that the samples derived from field dissipation studies are the same as described in the residue method for fluazinam in soil [Ryan, J., Sapiets, A. (1992)].

Findings:

Validation data are summarised in Table B.5.3.

No interferences were observed at the retention time of fluazinam above 30% of the LOQ.

The calibration curve (seven levels) in the range of 0.01 to 0.5 µg/mL is performed but the correlation coefficient and equation of the calibration graph is missing. The LOQ can be set to 0.01 mg/kg.

Conclusion:

A confirmatory technique is required and the correlation coefficient and equation of the calibration graph is missing.

B.5.3.2 Water (including drinking water) (Annex IIA 4.2.3, Annex IIIA 5.2.3)

Drinking water:

Reference: Ryan, J., Sapiets, A. (1994): Fluazinam: Validation of a Method for the

Determination of Residues in Drinking Water. Zeneca Agrochemicals, unpublished, (Report No. RJ 1576B)

Guideline: None specified. This report provides validation of the method to be used to determine residues of IKF-1216 in water samples

GLP: no certificate available but GLP compliance is not necessary.

Validity: The method is considered acceptable, but a confirmatory technique is missing.

Principle of the method:

Tap water is filtered under vacuum through Empore™ extraction disks. Fluazinam residues are eluted from the disks with ethyl acetate. The eluate is evaporated to dryness, dissolved in acetone and analyzed by capillary GLC using Restek RT_x capillary column 15 m x 0.32 mm ID, 0.5 µm film thickness.

The tap water was available at Jealott's Hill Research Station.

Findings:

Validation data are summarised in Table B.5.3.

No interferences were observed at the RT of fluazinam above 30% of the LOQ.

The linearity range is performed 0.02 to 0.5 µg/L with a correlation coefficient of 0.997, which covers sufficiently the fortification levels of 0.10 to 1.00 µg/L (calculated according to the provided analytical procedure), therefore the LOQ is set to 0.10 µg/L.

Conclusion:

A confirmatory technique is required.

Surface water:

Reference: Wais, A. (2001): Validation of A Residue Analytical Method for Fluazinam (IKF-1216) in Surface Water. RCC Ltd, unpublished, (Report No. 823803)

Guideline: -Residue Analytical Method, Commission Directive 96/46/EC, July 16, 1996, European Commission, Guidance Document on Residue Analytical Methods, SANCO/825/00 rev. 6, Jun. 20, 2002, SANCO/3029/99 rev. July 11, 2002 - Working document. This report provides validation of the method to be used to determine residues of IKF-1216 in surface water samples

GLP: yes

Validity: The method is considered acceptable.

Principle of the method:

To fifty mL of surface water (River "Wiese", D-79540 Lorrach, Germany)*) ten mL of dichloromethane are added. The mixture is transferred into a 125-mL separatory funnel. The flask previously containing the mixture is rinsed using another 10 mL of dichloromethane and the rinsing is added to the mixture in the separatory funnel. The funnel is shaken for about 2 min. After separation of the layers, the dichloromethane layer (bottom layer) is drained into a 100-mL round bottomed flask. The liquid-liquid extraction is then repeated using another 20 mL of dichloromethane. The combined organic extracts are evaporated to dryness under vacuum at about 25 ºC using a rotary evaporator. The dry residue is dissolved in 2 mL of

acetonitrile using an ultrasonic bath. Another 2 mL of water are added (final volume: 4 mL).
All samples are analyzed by HPLC –UV [column: Hypersil C18 BDS; 3µm; 100 mm x 4.6 mm; eluent: water/acetonitrile (30/70 v/v) + 0.1% TFA] at 240 nm.
HPLC-DAD determination was used as confirmation at 265 nm (second maximum).
*) TOC: 2400 mg C/L; pH: 7.4; hardness: 9 °dH; residue of evaporation 200 mg/L.

Findings:

Validation data are summarised in Table B.5.3.
No interferences were observed at the RT of fluazinam above 30% of the LOQ (10 µg/L).
The calibration was performed using standards in the range of 0.05 µg/mL to 5 µg/mL with a correlation coefficient of 1.000 and 0.999 for the confirmatory method respectively.

Conclusion:

The method is sufficiently validated in accordance to guidance doc. SANCO 825/00.
But due to the fact that the relevant residue limit (NOEC for fish) is 2.9 µg/L for fluazinam in surface water, a method with an adequate LOQ is required.

Method for residues in freshwater:

Reference: Shults, S. K., Brock, A. W., Laveglia, J. (1995): Technical Fluazinam (IKF-1216) – The Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. Ricerca, (Report No. 5107-92-0035-TX-002)

Guideline: --

GLP: no (for the added analytical method)

Validity: The validation of the method is not in accordance with guidance doc. SANCO 825/00.

Principle of the method:

Method validation/recovery samples were prepared in freshwater. The aqueous samples were fortified with fluazinam to produce fortification levels of 1 µg/L, 10.0 µg/L, 100 µg/L and 400 µg/L. The samples were extracted two times with hexane. After each addition of hexane, the sample was shaken for approximately two minutes by hand and the phases allowed to separate. The organic (hexane) phase was drained through anhydrous sodium sulphate. The dried extract was collected into an appropriately sided round bottom flask, isooctane was then added and the volume of the extract was reduced to appropriate isooctane volume (0.5%) using rotary evaporation. The remaining residue was adjusted with the appropriate volume of hexane and an aliquot was analyzed by GC-ECD (DB 608 15 m × 0.53 mm I.D., 1.0 µm).

Findings:

Calibration data are demonstrated by a linearity graph in the range of 20 to 200 µg/L (4 levels in duplicate), and a correlation coefficient of 0.993.
Under Sample Fortification is mentioned that levels of 400, 100, 10.0 and 1.00 µg/L were produced.

In Appendix VI (statistical analysis), nominal concentrations of 1.25, 5.0 and 20 µg/L are indicated.

Analytical results for the recovery are presented in Table B.5.3.

Conclusion:

A detailed validation according to guidance document SANCO 825/00 is required as no fortification level reveals a LOQ of 0.0954 µg/L and the sample number is not sufficient (3 samples instead of 5). Additional clarification about the concentration of the fortification levels is required.

A confirmatory technique is missing.

B.5.3.3 Air (Annex IIA 4.2.4, Annex IIIA 5.2.4)

Reference: Ryan, J., Sapiets, A. (1993): Fluazinam: Validation of a Stepped Model to Determine Residues in Air. Zeneca Agrochemicals, unpublished, (Report No. RF1529B)

Guideline: None specified

GLP: no certificate available, GLP compliance is not necessary.

Validity: The method is considered not acceptable as no linearity data are provided.

Principle of the method:

Fluazinam is adsorbed by suction of air (2 L/minute for 6 hours) through Tenax sampling tubes containing two layers (50 and 100 mg) of the adsorbent separated by a glass wool plug. After elution by acetone the solution is evaporated to dryness and re-diluted in Acetonitrile/water (60:40 v/v) + 0.4% acetic acid. Analysis for fluazinam was by HPLC with UV detection [column: Spherisorb 5 ODS2, 15 cm x 4.6 mm ID; mobile phase: Acetonitrile/water (60:40 v/v) + 0.4% acetic acid] at 258 nm.

The experiment was carried out at 20 °C and 45% relative humidity and 35 °C and 80% relative humidity. The adsorption of the sampling tubes and the stability of fluazinam once adsorbed onto the sorbent and then stored at -15 ºC for four weeks was determined at both fortification levels.

Findings:

The method is not evaluated by RMS as the method is not considered valid according to guidance document SANCO 825/00.

No linearity data have been provided.

Conclusion:

The method is not valid as no linearity data are included.

Table B.5.3 Validation data for analytical methods for the determination of residues in soil, water, air

References	Detection method	Determined analyte	Matrix	LOQ [mg/kg]	Fortification level [mg/kg]	Average recovery [%]	RSD [%]	n
Ryan, J., Sapiets, A. (1992) (ARAM 211)	GC-ECD	Fluazinam	Soil	0.05	0.01 0.02 0.05 0.10 0.15 0.20 0.40 0.50 0.75	75.8* 85.9* 90.1* 89.1* 100* 91.4* 104* 88.8* 93*	4.4* 13.3* 10.5* 9.8* 11.3* 10.2* 7.0* 10.2* 3.0*	4 10 11 10 2 8 4 5 2
Burke, S., Sapiets, A., Gentle, W. (1993) (RAM/218/01)	HPLC-UV	HYPA	Soil (Study 92JH136/A)	0.01	0.01 0.02 0.05	89.8* 82.1* 86.5*	4.2* 10.3* 9.6*	6 14 8
			Soil (Study 91JH108)	0.01	0.01 0.02 0.05 0.10 0.20	91.5* 82.8* 83.3* 81.0* 83.8*	32.9* 22.4 5.2* 10.9* 3.0*	4 5 4 4 4

References	Detection method	Determined analyte	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Average recovery [%]	RSD [%]	n
Ryan, J., Sapiets, A. (1994) (RJ 1576B)	GC-ECD	Fluazinam	Drinking water	0.10	0.05 0.10 0.50 1.00	107.3* 99.3* 87.3* 91.5*	6.1* 12.5* 3.5* 6.5*	4 3 4 4
Wais, A. (2001) (823803)	HPLC-UV	Fluazinam	Surface water	10.0	10.0 100.0 400.0	99.2 99.9 98.1	2.6 3.7 1.5	5 5 5

References	Detection method	Determined analyte	Matrix	LOQ [µg/L]	Fortification level [µg/L]	Average recovery [%]	RSD [%]	n
	HPLC-DAD (conf.)				10.0 100	100.2 97.4	1.95	3 1
Shults, S. K., Brock, A. W., Laveglia, J. (1995) (5107-92-0035-TX-002)	GC-ECD	Fluazinam	Fresh water	1.0	1.0 10.0 100 400	99.3* 107.9* 105.6* 97.6*	11.8* 8.9* 6.6* 6.2*	3 3 3 3

* calculated by RMS since only means and RSDs over all fortification levels are provided or no calculation has been provided
marked grey values are not covered by the linearity range

B.5.4 Analytical methods (residue) for body fluids and tissues (Annex IIA 4.2.5, Annex IIIA 5.2.5)

B.5.4.1 Body fluids and tissues

Reference: Kenyon, R. G. (1994): Analytical Procedure for the Determination of Fluazinam Residues in Cow Muscle and Liver. Ricerca, Inc., unpublished, (Report No. 6068-94-0088-MD-001)

Guideline: This study was conducted according to Guide 171-4 (d)

GLP: yes

Validity: The method is considered not acceptable.

Principle of the method:

For liver, an aliquot of the methanol extract (equivalent to 10 g of sample) was filtered, acidified with 300 ml of 0.2N HCl and partitioned with twice hexane (100 and 50 ml). The aqueous portion was discarded and the hexane phase was then partitioned twice with 150 ml of 0.5N NaOH solution. The hexane was discarded, the aqueous phase was acidified at pH below 1 with concentrated HCl and partitioned twice with hexane (100 and 50 ml). The hexane phase remaining was evaporated to dryness and the residue dissolved in 1:1 methylene chloride:cyclohexane (v/v), then cleaned up by gel permeation chromatography (GPC).

For muscle (either ground chuck or porterhouse steak), an aliquot (100 ml) of the methanol extract was diluted with water, acidified with concentrated HCl and partitioned twice with petroleum ether. The petroleum ether fraction was evaporated to dryness and the residue dissolved in 1:1 methylene chloride:cyclohexane (v/v), then cleaned up by GPC.

After GPC cleanup of each matrix fraction, the samples were reconstituted in hexane. An aliquot was passed through a Florisil cleanup column prior to analysis by GC-ECD (column: J&W DB-608 megabore 15 m 0.53 mm ID, 0.83 µm film thickness)

Findings:

The method is not evaluated by RMS as the method is not considered valid according to guidance document SANCO 825/00.

Conclusion:

The method is not valid as no linearity data are included.

No confirmatory method is reported.

Reference: Tillkes, M. (1995): Validation of DFG Method S 19 (Modified Extraction) for the Determination of Residues of Fluazinam in Milk, Muscle, Kidney, Liver and Egg. Dr. Specht & Partner, Chemische Laboratorien GMBH, unpublished, (Report No. ZEN-9504V)

Guideline: This study was conducted using DFG Method S 19 from the Manual of Pesticides Residue Analysis DFG

GLP: yes

Validity: The method is considered not acceptable.

Principle of the method:

For validation of the method, untreated samples of milk, muscle, kidney, liver and egg were fortified with fluazinam to obtain a residue content of 0.02 and 0.2 mg/kg. Fortified samples, as well as the corresponding untreated samples, were analyzed for fluazinam according to test method DFG S 19.

Sample material is extracted with acetone after addition of sufficient water so that the acetone:water ratio remains constant at 2:1 (v/v) during the extraction procedure. The extract is saturated with NaCl and mixed thoroughly. Ethyl acetate/cyclohexane (1:1 v/v) is used for liquid-liquid partitioning. An aliquot of the organic phase after evaporation is cleaned up by gel permeation chromatography on Bio Beads S-X3 polystyrene gel using ethyl acetate/cyclohexane (1:1 v/v) as eluant. The residue-containing fraction is concentrated, cleaned up on a small silica gel column, and analyzed by GC-ECD using a capillary column (DB-1 30m 0.25 ID 0.25 µm film thickness).

Findings:

The method is not evaluated by RMS as the method is not valid.

Conclusion:

The method is not valid as no linearity data are included.

No confirmatory method is reported.

B.5.5 Evaluation and assessment

B.5.5.1 Active substance and formulation analysis

Adequate analytical methods has been provided for the determination of fluazinam in the technical material [HPLC-UV] and in the representative formulation as well as for the determination of the significant and relevant impurities in the technical material [HPLC-UV].
A suitable method for the determination of the relevant impurity 5 in the formulation has been submitted.
According to Section Toxicology impurity 6 is also considered to be relevant, therefore an analytical method for the determination in the formulation must be provided.

No CIPAC methods exist at the moment of evaluation for the determination of fluazinam in the compound as manufactured and in the formulation.

B.5.5.2 Residue analysis

The analytical method for residues in food/feed of plant origin is successfully validated since the LOQs enable the enforcement of the relevant residue limits (at the time of evaluation). Mean recovery rates at each fortification level are in the range of 70 to 110 % with a relative standard deviation of ≤ 20 %. The analytical calibration extend over a range appropriate to the lowest and highest nominal concentration of the analyte ± at least 20% for this analytical method. No interfering blanks (< 30 % of the LOQ) occurred. The linearity range of the ILV needs clarification.
Readily available equipment and reagents are used.

Matrix	LOQ	Relevant residue limit	Principle of the method
Plant and plant products	Potatoes (grape and wine) 0.01 mg/kg	Potatoes 0.01 mg/kg	GC-ECD
Soil (fluazinam)	0.05 mg/kg*)	0.05 mg/kg general upper limit	GC-ECD
Soil (HYPA)	0.01 mg/kg	6.1 mg/kg (most sensitive organism: Collembola)	HPLC-UV
Drinking water	0.1 µg/L*)	0.1 µg/L EU drinking water limit	GC-ECD
Surface water (fluazinam)	10 µg/L	2.9 µg/L NOEC (fish)	HPLC-UV

Matrix	LOQ	Relevant residue limit	Principle of the method
Air	a new validation is required	1.1 µg/m^3 based on an $AOEL_{systemic}$ of 0.0035 mg/kg b.w./d	HPLC-UV

*) For the determination of residues in soil and drinking water the LOQs are set by RMS since validation data are not acceptable on all points for the lower LOQs proposed by the notifier.

Methods for the determination of residues in soil and drinking water needs additional confirmatory techniques and a new validation for the determination of residues in air is required the same as for the analytical methods (residue) for body fluids and tissues.

According to Section Residue, a method for monitoring TFAA is necessary, if a residue definition is set for TFAA (trifluoro acetic acid) in rotational crops.

Due to the fact that the relevant residue limit is 2.9 µg/L for fluazinam in surface water, a valid method with an adequate LOQ is required.

The submitted methods for the determination of residues of fluazinam in body fluids and tissues are not sufficiently validated due to the lack of linearity data and therefore a validation is required.

B.5.6 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					
IIA, 4.2.1/02	Ryan J., Sapiets, A.	1991	The Determination of Residues of Fluazinam in Crops, a gas-liquid chromatography method. ICI Agrochemicals, Report No. ARAM 87/1. Not GLP, unpublished	N	ISK
IIA, 4.2.1/03	Schulz H.	1992	Determination of the Residues of IKF-1216 (Fluazinam) and Its Metabolites in Grapes, Wine and Potatoes; Amendments 7, 8, 9 and 10. RCC Umweltchemie AG, Report No. 284670 GLP, unpublished	N	ISK
IIA, 4.2.1/04	Wais, A.	2002	Validation of the Residue Analytical Method for Fluazinam in Potato (RAC Tubers), Grapes and Wine RCC Ltd., Report No. 845972 GLP, unpublished	Y	ISK
IIA, 4.2.1/06	Wais, A.	2005	1st Amendment to compilation report Validation of the Residue Analytical Method for Fluazinam in Potato (RAC Tubers), Grapes and Wine RCC Ltd., Report No. 845972 GLP, unpublished	Y	ISK
IIA, 4.2.1/05	Brachet, A., Nésa, C.	2002	Independent Laboratory Validation (ILV) of the Analytical method RCC-845972 for the Determination of Residues of Fluazinam (IKF-1216) in Potato Battelle, Report No. A22-02-04 GLP, unpublished	Y	ISK
IIA, 4.2.1/07	Specht, W.	1992	Überprüfung der Anwendbarkeit der DFG-multimethode S 19 für die Rückstands-bestimmung von fluazinam in Kartoffeln Dr. Specht and Partner Chemische Laboratorien Report No. 92856/92 GLP, unpublished	N	ISK
IIA, 4.2.2/01	Ryan, J., Sapiets, A.	1992	The Determination of Residues of Fluazinam in Soil (A gas liquid chromatography method). ICI Agrochemicals, Report No. ARAM 211 GLP, unpublished	N	ISK
IIA, 4.2.2/03	Sapiets, A.	2005	Statement on the analytical methods for soil Syngenta, no report number, Non-GLP, unpublished	Y	ISK

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
IIA, 4.2.2/02	Burke, S., Sapiets, A., Gentle, W.	1993	The Determination of the Fluazinam Metabolite "HYPA" in Soil, A High Performance Liquid Chromatography Method. ICI Agrochemicals, SOP No. RAM/218/01 GLP, unpublished	N	ISK
IIA, 4.2.3/01	Ryan, J., Sapiets, A.	1994	Fluazinam: Validation of a Method for the Determination of Residues in Water. Zeneca Agrochemicals, Report No. RJ 1576B GLP, unpublished	N	ISK
IIA, 4.2.3/02	Ryan, J., Sapiets, A.	1992	The Determination of Residues of Fluazinam in Water. ICI Agrochemicals, Report No. ARAM 210 GLP, unpublished	N	ISK
IIA, 4.2.3/03	Wais, A.	2001	Validation of A Residue Analytical Method for Fluazinam (IKF-1216) in Surface Water. RCC Ltd, unpublished, Report No. 823803 GLP, unpublished	Y	ISK
IIA, 4.2.3	Shults, S. K., Brock, A. W., Laveglia, J.	1995	Technical Fluazinam (IKF-1216)– The Chronic Toxicity to the Fathead Minnow (*Pimephales promelas*) During a Full Life-Cycle Exposure. Generated by: Springborn Laboratories Report No. 5107-92-0035-TX-00 GLP / GEP: yes unpublished	N	ISK
IIA, 4.2.4/01	Ryan, J., Sapiets, A.	1993	Fluazinam: Validation of a Stepped Model to Determine Residues in Air. Zeneca Agrochemicals, Report No. RF1529B GLP, unpublished	N	ISK
IIA,4.2.5/01	Kenyon, R. G.	1994	Analytical Procedure for the Determination of Fluazinam Residues in Cow Muscle and Liver. Ricerca, Inc., Report No. 6068-94-0088-MD-001 GLP, unpublished	N	ISK
IIA,4.2.5/02	Tillkes, M.	1995	Validation of DFG Method S 19 (Modified Extraction) for the Determination of Residues of Fluazinam in Milk, Muscle, Kidney, Liver and Egg. Dr. Specht & Partner, Chemische Laboratorien GMBH, Report No. ZEN-9504V GLP, 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
Annex III					
IIIA, 5.1.1/01	Krainz, A.	2001	Validation of an Analytical Method for the Determination of Fluazinam (Active Ingredient) in IKF-1216 500SC. RCC Umweltchemie AG, Report No. 838923 GLP: yes unpublished	Y	ISK
IIIA, 5.1.3/01	Tandy, M. J.	1996	The Determination of Fluazinam in Technical and Formulated Materials by High Performance Liquid Chromatograph. Zeneca Agrochemicals, Jealott's Hill Research Station, Report No. PAM 600 GLP: no unpublished	N	ISK