Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Fluazinam

EC Number: -

CAS Number: 79622-59-6

ECHA/RAC/CLH-O-0000002667-66-01/A1

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

15 June 2012

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Fluazinam

EC Number: -

CAS Number: 79622-59-6

Index Number:

Contact details for dossier submitter:

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Austria

Version number: 2 **Date:** **03. 2011**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Fluazinam
EC number:	-
CAS number:	79622-59-6
Annex VI Index number:	-
Degree of purity:	960 g/kg
Impurities:	5-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- α,α,α-trifluoro-4,6-dinitro-o-toluidine Max. content: 2.0 g/kg For other impurities see DAR, Vol 4, Confidential

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	Regulation (EC) No 1272/2008	**Directive 67/548/EEC**
Current entry in Annex VI to CLP Regulation	-	-
Proposal by dossier submitter for consideration by RAC	Skin Irrit. 2 (H315) Skin Sens. 1 (H317) Eye Dam. 1 (H318) Acute Tox. 4 (H332) STOT SE 3 (H335) Repr. 2 (H361) Aquatic Acute 1 (H400)	Xn; R20 Xi; R37/38 Xi; R41 R43 Repr. Cat. 3, R63 N; R50/53

	Acute M-factor = 10 Aquatic Chronic 1 (H410)	
Resulting harmonised classification (future entry in Annex VI to CLP Regulation) as proposed by dossier submitter	Skin Irrit. 2 (H315) Skin Sens. 1 (H317) Eye Dam. 1 (H318) Acute Tox. 4 (H332) STOT SE 3 (H335) Repr. 2 (H361) Aquatic Acute 1 (H400) Acute M-factor = 10 Aquatic Chronic 1 (H410)	Xn; R20 Xi; R37/38 Xi; R41 R43 Repr. Cat. 3, R63 N; R50/53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Directive 67/548/EEC:

Symbols: Xn, Xi, N
Risk phrases: R20, R37/38, R41, R43; R50/53
Repr. Cat.3, R63

Safety phrases: S2, S13, S20/21, S24/25, S26, S27/28, S36/37/39, S38, S45, S63, S56, S57, S60, S61

Regulation EC 1272/2008:

Signal words: Warning, Danger

Symbols: 

Hazard statements: H332, H335, H315, H318, H317, H361, H400, H410

Precautionary statements: P201, P202, P261, P264, P270, P271, P272, P273, P280, P281, P501

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Conclusive, but not sufficient for classification
2.2.	Flammable gases				Conclusive, but not sufficient for classification
2.3.	Flammable aerosols				Conclusive, but not sufficient for classification
2.4.	Oxidising gases				Conclusive, but not sufficient for classification
2.5.	Gases under pressure				Conclusive, but not sufficient for classification
2.6.	Flammable liquids				Conclusive, but not sufficient for classification
2.7.	Flammable solids				Conclusive, but not sufficient for classification
2.8.	Self-reactive substances and mixtures				Data lacking
2.9.	Pyrophoric liquids				Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids				Inconclusive
2.11.	Self-heating substances and mixtures				Inconclusive
2.12.	Substances and mixtures which in contact with water emit flammable gases				Conclusive, but not sufficient for classification
2.13.	Oxidising liquids				Conclusive, but not sufficient for classification
2.14.	Oxidising solids				Conclusive, but not sufficient for classification
2.15.	Organic peroxides				Conclusive, but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals				Conclusive, but not sufficient for classification
3.1.	Acute toxicity - oral	no			conclusive, but not sufficient for classification

	Acute toxicity - dermal	no			conclusive, but not sufficient for classification
	Acute toxicity - inhalation	Cat. 4, H332		Cat. 4, H332	
3.2.	Skin corrosion / irritation	Cat. 2, H315		Cat. 2, H315	
3.3.	Serious eye damage / eye irritation	Cat. 1, H318		Cat. 1, H318	
3.4.	Respiratory sensitisation				conclusive, but not sufficient for classification
3.4.	Skin sensitisation	Cat. 1, H317		Cat. 1, H317	
3.5.	Germ cell mutagenicity				conclusive, but not sufficient for classification
3.6.	Carcinogenicity				conclusive, but not sufficient for classification
3.7.	Reproductive toxicity	Cat. 2, H361		Cat. 2, H361	
3.8.	Specific target organ toxicity –single exposure	Cat. 3, H335			
3.9.	Specific target organ toxicity – repeated exposure				conclusive, but not sufficient for classification
3.10.	Aspiration hazard				conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1 - H400 Aquatic Chronic 1 - H410.	M-Factor 10		
5.1.	Hazardous to the ozone layer				Data lacking

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal words:	Warning, Danger
Hazard statements:	H332, H335, H315, H318, H317, H361, H400, H10
Precautionary statements:	P261, P264, P270, P271, P272, P280, P281, P273, P391, P501
Suppl. Hazard:	EUH401

Proposed notes assigned to an entry: -

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	-	-	-	Conclusive, but not sufficient for classification
Oxidising properties				Conclusive, but not sufficient for classification
Flammability				Conclusive, but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*				-
Thermal stability				Conclusive, but not sufficient for classification
Acute toxicity	Xn, R20		Xn, R20	
Acute toxicity – irreversible damage after single exposure	no			conclusive, but not sufficient for classification
Repeated dose toxicity	no			conclusive, but not sufficient for classification
Irritation / Corrosion	Xi, R37/38, 41		Xi, R38, R41	
Sensitisation	Xi, R43		Xi, R43	
Carcinogenicity	no		no	conclusive, but not sufficient for classification
Mutagenicity – Genetic toxicity	no		no	conclusive, but not sufficient for classification
Toxicity to reproduction – fertility				conclusive, but not sufficient for classification
Toxicity to reproduction – development	Xn, R63		Xn, R63	
Toxicity to reproduction – breastfed babies. Effects on or via lactation				conclusive, but not sufficient for classification
Environment	N; R50/53			

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Symbols: Xn, Xi, N

R-phrases: R 20; R37/38, R41, R43; R63, R50/53
S-phrases: S2, S13, S20/21, S24/25, S26, S27/28, S36/37/39, S38, S45, S56, S57, S60, S61, S63

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Fluazinam is a pyridine fungicide with protective action with activity against fungi from the class of *Oomycetes*. In 2008 it was approved for Annex I listing as a third stage Part A Review compound under Council Directive 91/414/EEC, with Austria as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, fluazinam should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physical and chemical properties, human health and environmental endpoints. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of fluazinam under Directive 91/414/EEC. This assessment was based on one full data package submitted by one company.
Fluazinam is not currently listed in Annex VI of Regulation EC 1272/2008 (CLP Regulation). Following evaluation of the data this proposal seeks to propose classification for health hazard and environment. No classification for physico-chemical properties is proposed. No disagreement on classification and labeling proposal were given between Austria as Rapporteur Member State and other Member States during the peer review procedure for Annex I inclusion..

2.2 Short summary of the scientific justification for the CLH proposal

For Fluazinam, no classification and labelling has been proposed regarding physical and chemical properties, neither by Rapporteur Member State (Austria) nor during the PRAPeR peer review.

Considering human health, fluazinam is of low acute toxicity with LD_{50} values $\geq$ 4100 mg/kg bw after oral application to mice and rats of both sex.
After acute dermal application of fluazinam to rats of both sex, the acute dermal LD_{50} was > 2000 mg/kg bw.
Inhalative LC_{50} of fluazinam in rats:
The original study design was whole body exposure (which might include oral, dermal and inhalation route, whole-body exposure) and inhalative LC_{50} of fluazinam was 0.46 mg/l. In the repeat study snout only exposure was used. Furthermore, Polyethylene glycol 400 was used as solvent control in the original study. As fluazinam is completely soluble in polyethylene glycol 400, the exposure results might have differences from that of representative exposure. In the repeat study, fluazinam was administered as a dust aerosol which is more representative of the potential exposure. The inhalative LC_{50} of fluazinam in rats (nose only exposure) was > 1.1 mg/l (acute hazard category 4, H332).
Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed in an acute inhalative toxicity study, so a classification according to Regulation EC 1272/2008 seems justified (acute hazard category 3, H335).
Repeated dermal administration of fluazinam to rats for 3 weeks revealed effects to the skin (acanthosis, dermatitis, scabs and ulceration) compared to controls. According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 2, H315.
Significant corneal epithelial effects involving up to approximately 25 % of the corneal surface in 3 rabbits at 72 hours were observed which persisted in 2 animals through day 7 of the study. Iridal effects were observed in 4 rabbits and persisted in one animal till termination on day 21. Conjunctival irritation was observed in all six rabbits at the 1 hour interval and persisted in one animal till day 21. So fluazinam is severely irritating to the eyes of New Zealand White rabbits (acute hazard category 1, H318).

In the Magnusson and Kligman dermal maximization study and in the Buehler-Test fluazinam caused evidence of delayed contact hypersensitivity in guinea pigs. According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 1, H317.
In the reproduction studies, fertility parameters and the offspring were not affected, but the indications of teratogenicity in the rat studies led to the proposal of hazard category 2 for reproductive toxicity, hazard statement H361 (Suspected of damaging the unborn child).

Regarding environment, classification as R50 and R53 (DSD) or H400 (CLP **aquatic environment hazards acute category 1** very toxic to aquatic organisms) and H410 (CLP **aquatic environment hazards chronic category 1** Very toxic to aquatic life with long lasting effects**)** are proposed.

H400 follows from the acute toxicity to fish (*Oncorhynchus mykiss* LC_{50}= 0.036 mg/L, Gelin & Laveglia 1992),

H410 is based on the rapid degradability in a water/wediment study with a DT_{50} whole sys.: <16 d (DT_{50} whole sys.: phenyllabelled: 4.3 d; pyridyllabelled: 4.6 d) and on the chronic toxicity to fish (*Pimephales promelas* (Shults et al. 1995)) $NOEC_{F0\ growth,\ F1\ survival}$= 0.0029 mg/L .

2.3 Current harmonised classification and labelling

Fluazinam has not been previously discussed at TC C&L (Dir. 67/548/EEC); no harmonised classification and labelling exists.

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No entry in Annex VI, Table 3.1.

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No entry in Annex VI, Table 3.2.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Not provided by the notifier

2.4.2 Current self-classification and labelling based on DSD criteria

No current self-classification and labelling based on DSD Regulation criteria

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification (Fluazinam is a pesticide).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

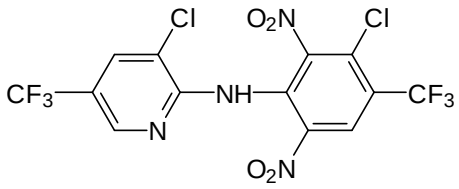
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	-
EC name:	Fluazinam
CAS number (EC inventory):	-
CAS number:	79622-59-6
CAS name:	3-chloro-N-[3-chloro-2, 6-dinitro-4-trifluoromethyl) phenyl]-5-(trifluoromethyl)-2-pyridinamine
IUPAC name:	3-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α- trifluoro-2, 6-dinitro-p-toluidine
CLP Annex VI Index number:	
Molecular formula:	$C_{13}H_4Cl_2F_6N_4O_4$
Molecular weight range:	465.1

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Fluazinam	>960 g/kg	No range, since minimal purity stated	-

Current Annex VI entry: no entry

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
5-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)- α,α,α-trifluoro-4,6-dinitro-o-toluidine	Max. content: 2.0 g/kg	-	-

For other impurities (confidential information) please refer to DAR – Vol 4 – conf.

Current Annex VI entry: no entry

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	-	-	-	-

Current Annex VI entry: no entry

1.2.1 Composition of test material

Physico-chemical properties: see table 9 (purity of tested technical material in the range from 90.0% to 99.7%)

Human health hazard assessment: purity of tested technical material in the range from 95.2 to 99.9 %

Environmental hazard assessment: purity of tested technical material in the range from 96.8 to 100 %

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

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.m³.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="2">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} [$L \cdot mol^{-1} \cdot cm^{-1}$]</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>
<tr><td colspan="3">ε above 290 nm in alkaline solution > 10</td></tr>
</table></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>λ_{max} [nm]</th><th>ε_{max} [$L \cdot mol^{-1} \cdot cm^{-1}$]</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>
<tr><td colspan="3">ε above 290 nm in neutral and alkaline solution > 10</td></tr>
</table></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>
</table>

<table>
<tr><th>Study</th><th>Method</th><th>Results</th><th>Conclusion/Comment</th><th>Reference</th></tr>
<tr><td></td><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>
<tr><td></td><td>Fourier–Transform ^{1}H - NMR-Spectroscopy
GLP</td><td>Purified product (purity: 99.8% w/w)</td><td>Acceptable
The NMR spectrum of fluazinam is in agreement with the chemical structure</td><td>van Rijsbergen, L.M. (2002) (Document 341156)</td></tr>
<tr><td></td><td>MS - Spectroscopy
MS/MS (API negative mode)
GLP</td><td>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</td><td>Acceptable
The MS-spectrum is consistent with the chemical structure</td><td>van Rijsbergen, L.M. (2002) (Document 341178)</td></tr>
<tr><td>B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)</td><td>MS (EI and CI), IR, ^{13}C - NMR and UV spectrum
GLP</td><td><u>Impurity 5:</u>
Purity 97.3%
Concentration: 0.45 mg/mL in acetonitrile
UV:<table><tr><th>$\lambda_{max\ [nm]}$</th><th>ε_{max} [$L \cdot mol^{-1} \cdot cm^{-1}$]</th></tr><tr><td>239</td><td>18893</td></tr></table></td><td>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</td><td>Bramstedt W.R., Kogovsek L.M. (1999) (Document 4039-98-0177-AS-001)</td></tr>
<tr><td></td><td></td><td><u>Impurity 6:</u></td><td>Spectra are missing. Data requirement see volume 1 level 4</td><td></td></tr>
</table>

Study	Method	Results	Conclusion/Comment	Reference
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)
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) 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	Acceptable	Sanders, J. (1993) (Document 4039-91-0384-AS-001)
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 conditic 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)

Study	Method	Results	Conclusion/Comment	Reference
B.2.1.17 Quantum yield (IIA 2.9.3)	Calculation	Quantum yield in moles degraded per Einstein absorbed $5.1x10^{-5}$ (pH 5 buffer) $1.7x10^{-5}$ (pH 6 distilled water) $2.1x10^{-6}$ (pH 9 buffer)	Acceptable For details see B 8.4 Fate and behaviour in water	Wadley, A.M. (1992) (Document RIC1726)
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 6.1×10^{-11} and 1.5×10^{-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 1.5×10^6 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)

Study	Method	Results	Conclusion/Comment	Reference
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)
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)	EEC/A17 GLP	Technical product (purity: 97.3% w/w) The maximum burning rate of the test substance/cellulose mixture was lower (0.81 mm/s) than the maximum burning rate of the $Ba(N0_3)_2$/ cellulose mixture (0.85 mm/s). Test substance is not oxidizing	Acceptable	Brekelmans M.J.C. (2006) (Report 460777)

According to Dir. 91/414/EEC, granulometry is not required for active substances. Thus, no study considering this end-point has been provided.

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

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.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

3.1 *[Insert hazard class when relevant and repeat section if needed]*

3.1.1 Summary and discussion

Based on the data made available, no classification and labelling is considered necessary.

3.1.2 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, no classification for Fluazinam considering physico-chemical properties is considered necessary. For details, please refer to table 9, summary of physico-chemical properties.

3.1.3 Conclusions on classification and labelling

No classification is required considering physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Metabolic and kinetic studies were conducted with radiolabeled fluazinam, following oral administration at a low dose of 0.5 mg/kg bw, a high dose of 50 mg/kg bw and 14 daily oral doses of unlabeled fluazinam followed by ^{14}C-fluazinam (labelled in the phenyl position) of 0.5 mg/kg bw. The majority of radiolabeled material was detected in the feces (> 88 %). Urine was a minor excretory route (2 - 4 %). Less than 1 % of the administered dose was found in the carcass. The highest concentration was detected in the liver. There were no major differences related to sex or dose level. The median peak time for blood concentration of radiolabel activity for both sexes was 6 hours. At the time of peak concentration, the radioactivity in the blood represented 0.4 % - 0.6 % of the administered dose for 0.5 and 50 mg/kg bw dose groups. By 72 hours, about 0.1 % of the administered dose was found in the blood of both sexes at both dose levels. Approximately 30 % (high dose) – 40 % (low dose) of fluazinam was considered to be absorbed based on excretion rates in bile and urine. The predominant route of excretion of the absorbed dose was the bile, which contained approximately 87 % of the absorbed dose. 24 hours after dose administration, biliary excretion of the absorbed dose was 80 % complete at the high dose level and 92 % complete at the low dose level.
Metabolites were identified using several techniques including HPLC coelution with standards, direct identification by mass spectrometry and comparison with standards, NMR, and degradation experiments. The distribution of these metabolites, as a function of dosing regimen, position of radiolabel, and sex, was determined. Major metabolites isolated and identified from feces, urine and bile were the parent compound, DAPA, AMPA, AMPA mercapturate, DAPA glucuronide and DAPA cysteine conjugate. The major metabolites of the organic fraction of feces were parent compound, AMPA and DAPA and the major metabolite in the aqueous fraction of feces was DAPA cysteine conjugate. The feces were the major route of excretion of fluazinam and its metabolites.

AMPA mercapturate, DAPA glucuronide and DAPA were found in the urine at low levels (≤ 2 % of administered dose) and AMPA mercapturate and DAPA glucuronide were found in the bile (≤ 5 % of administered dose). Fluazinam was also metabolized by the intestine microflora to form AMPA and DAPA. The identified metabolites were the same in samples from both phenyl and pyridyl labels, indicating that metabolic cleavage of the two rings did not occur. The metabolism of fluazinam was similar between male and female rats within a dose group. It can be concluded that fluazinam is metabolized by both reduction and glutathione conjugation and further metabolism.

4.1.2 Human information

No information available from case reports, epidemiological studies, medical surveillance, reporting schemes and national poisons centres.

4.1.3 Summary and discussion on toxicokinetics

The rate and extent of absorption of fluazinam was 30 % - 40 %, based on excretion rates in bile and urine (rat studies, 0.5 and 50 mg fluazinam/kg bw/d).
Distribution: Highest levels were found in the liver. There was no evidence for accumulation.
The rate and extent of excretion was rapid, mainly via feces (> 84 % within 24 h, > 93 % after 7 days).
Fluazinam was almost completely metabolized in animals by hydroxylation, followed by conjugation.

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity	CD-1 mice: m/f > 5000 mg/kg bw	Decreased motor activity	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat: m/f > 5000 mg/kg bw	Decreased motor activity, hunched posture, piloerection, ataxia	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat: m 4500 mg/ kg bw f 4100 mg/ kg bw	Hunched posture, piloerection, lethargy, diarrhoea	*Liggett, 1988*
Acute dermal toxicity	Sprague Dawley Rat: m/f > 2000 mg/kg bw	No reaction to treatment	*Cummins, 1988*
Acute inhalation toxicity	Sprague Dawley Rat: m: 0.463mg/l air f : 0.476 mg/l air (4h, whole body exposure)	Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea. Deaths due to respiratory failure.	*Tobeta, 1988*
Acute inhalation toxicity	Crl:CD (SD) rats: m/f > 1.1 mg/l air (4h, nose only exposure)	Dark red discoloration of the lungs.	*Kirkpatrick, 2006*

4.2.1 Non-human information

Acute toxicity: oral
After oral application to mice and rats of both sex, fluazinam is of low acute toxicity with LD50 values > 4100 mg/kg bw. Signs of toxicity were decreased motor activity, hunched posture, piloerection, ataxia.
For further details, please see Draft Assessment Report.

4.2.1.1 Acute toxicity: inhalation

Acute inhalation toxicity test of fluazinam technical in rats:

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

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

GLP: yes

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

Findings:
Clinical signs and mortality: During exposure, all animals showed reduced spontaneous movement, moist fur, nasal blot, cloudy eyeballs, decreased respiratory rate and gasping or abnormal breathing sound. Mortality occurred in males within 7 days after exposure and in females within 4 days after exposure.
Pathology: Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered mostly due to respiratory failure. Necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities.

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

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

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

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

Acute inhalation toxicity study of fluazinam technical in albino rats

Reference: *Kirkpatrick D.; 2006; S*tudy No. WIL-282007

Guideline: The study was conducted according to U.S. EPA OPPTS Guideline 870.1300 and OECD Guideline 403.

GLP: yes

Material and Methods:
Fluazinam technical (lot number A629/1995; purity 97.3 %) was administered to a group of 5 male and 5 female rats (strain: Crl:CD (SD); source: Charles River Lab. North Carolina). The animals weighted between 188 and 347 g and were 8 to 9 weeks old. Administration was for four hours via nose only exposure as a dust aerosol at a concentration of 1.1 mg/l. The mass median aerodynamic diameter (MMAD) was 2.2 + 2.49 µm (+ geometric standard deviation). Animals were observed for mortality and clinical signs during exposure, immediately following exposure on study day 0 and twice daily thereafter for 14 days. Body weights were recorded immediately before exposure (day 0) as well as 7 and 14 days after exposure. At the end of the 14-day observation period, all surviving rats were exsanguinated and necropsied. Animals dying during the study were necropsied immediately after death was noted.

Findings:
Clinical signs and mortality: Two males died during exposure. Significant clinical observations immediately following exposure included rales, closure of the eyes and red material around the eyes, nose and mouth. Clinical observations during the 14 days observation period consisted of rales, decreased defecation and urination and red material around the eyes, nose and mouth. All surviving animals were considered normal by study day 6 and surpassed their initial body weight by study day 14.
Pathology: Macroscopic finding noted for 1 male that died was dark red discoloration of the lungs. Necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities.

Conclusion:
Based on the results of this study, the acute inhalation LC_{50} (4 hour snout only exposure) of fluazinam was > 1.1 mg/l for male and female rats.

4.2.1.2 Acute toxicity: dermal

After acute dermal application of fluazinam to rats of both sex, the acute dermal LD50 was > 2000 mg/kg bw. There were no deaths and no reaction to treatment. There was no evidence of local irritation at the site of application.
For further details, please see Draft Assessment Report.

4.2.1.3 Acute toxicity: other routes

No data

4.2.2 Human information

No data

4.2.3 Summary and discussion of acute toxicity

After oral application to mice and rats of both sex, fluazinam is of low acute toxicity with LD_{50} values $\geq$ 4100 mg/kg bw.
After acute dermal application of fluazinam to rats of both sex, the acute dermal LD_{50} was > 2000 mg/kg bw.
Inhalative LC_{50} of fluazinam in rats:
The original study design was whole body exposure (which might include oral, dermal and inhalation route, whole-body exposure) and inhalative LC_{50} of fluazinam was 0.46 mg/l. In the repeat study snout only exposure was used. Furthermore, Polyethylene glycol 400 was used as solvent control in the original study. As fluazinam is completely soluble in polyethylene glycol 400, the exposure results might have differences from that of representative exposure. In the repeat study, fluazinam was administered as a dust aerosol which is more representative of the potential exposure. The inhalative LC_{50} of fluazinam in rats (nose only exposure) was > 1.1 mg/l.

4.2.4 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as harmful by inhalation (hazard symbol Xn, risk phrase R20) and acute hazard category 4 for inhalation exposure and labeled with signal word “Warning” and hazard statement H332 (Harmful if inhaled), respectively since the LC50 in rats is reported to be > 1.1 mg/l.

4.2.5 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as harmful by inhalation (hazard symbol Xn, risk phrase R20).

According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 4 for inhalation exposure and labeled with signal word “Warning” and hazard statement H332 (Harmful if inhaled).

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

After oral application to mice and rats of both sexes, fluazinam is of low acute toxicity with LD_{50} values ≥ 4100 mg/kg bw.
After acute dermal application of fluazinam to rats of both sexes, the acute dermal LD_{50} was > 2000 mg/kg bw.
Inhalation LC_{50} of fluazinam in rats: The original study design was whole body exposure (which might include oral, dermal and inhalation route) and inhalation LC_{50} of fluazinam was 0.46 mg/l. In the repeat dose study, snout only exposure was used. Furthermore, Polyethylene glycol 400 was used as solvent control in the original study. As fluazinam is completely soluble in polyethylene glycol 400, the exposure results might differ from that of representative exposure. In the repeat study, fluazinam was administered as *a dust aerosol* which is more representative of the potential exposure. The inhalation LC_{50} of fluazinam in rats (nose only exposure) was > 1.1 mg/l.
According to the classification criteria in Directive 67/548/EEC, fluazinam has to be classified as harmful by inhalation with Acute Tox. 4 (H332; "Harmful if inhaled") according to the CLP Regulation (Xn; R20 according to Directive 67/548/EEC) since the LC_{50} in rats is reported to be > 1.1 mg/l.

Comments received during public consultation

Four MSCAs made comments.
- Three MSCAs agreed with the proposal.
- One MSCA disagreed because it could not be concluded from the study that the exact LC_{50} would be below 5 mg/l.

During the RAC consultation of the 1st Draft Opinion, one RAC member agreed with the proposed classification and requested to add the additional labeling for corrosive effects to the respiratory tract (EUH071), based on destruction of the respiratory tract tissue observed in the third study and supported by the corrosive effects on eyes.

RAC assessment - comparison with the classification criteria and justification

Comparison with the classification criteria:

According to the CLP criteria for oral and dermal acute toxicity, if the LD_{50} values are above 2000 mg/kg bw, no classification and labelling is required.
Hence, when comparing the values observed for fluazinam in the acute oral and dermal toxicity studies with the criteria, no classification and labelling is necessary.
For dust, category 4 is defined to be for a range of exposure estimates between 1 and 4.5 mg/l.
Hence, as for fluazinam the inhalation LC_{50} of fluazinam in rats (nose only exposure to dust) was > 1.1 mg/l, Acute Tox. 4 (H332; "Harmful if inhaled") is justified.
In relation to EUH071 (additional labelling element) the proposal was that this would not be warranted because there were no signs of corrosivity in the acute toxicity inhalation study. This was supported by the RAC members.

Conclusion:

When comparing the available data with the classification criteria, RAC concluded that classification of fluazinam as Acute Tox. 4 (H332) according to the CLP Regulation and as Xn; R20 according to Directive 67/548/EEC was justified.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

In the first acute inhalative study (*Tobeta, 1988*), all animals showed reduced spontaneous movement, moist fur, nasal blot, cloudy eyeballs, decreased respiratory rate and gasping or abnormal breathing sound during exposure. Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered mostly due to respiratory failure.
In the second study (*Kirkpatrick, 2006*), significant clinical observations immediately following exposure included rales, closure of the eyes and red material around the eyes, nose and mouth. Clinical observations during the 14 days observation period consisted of rales, decreased defecation and urination and red material around the eyes, nose and mouth. All surviving animals were considered normal by study day 6 and surpassed their initial body weight by study day 14. Macroscopic finding noted for 1 male that died was dark red discoloration of the lungs. Necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities.

4.3.2 Comparison with criteria

In the first acute inhalative study (*Tobeta, 1988*) signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered mostly due to respiratory failure. In the second study (*Kirkpatrick, 2006*) macroscopic finding noted for 1 male that died was dark red discoloration of the lungs. In both studies, necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities. Therefore, according to Regulation EC 1272/2008, classification for STOT SE Specific target organ toxicity –single exposure: Cat. 3, H335 is required. According to DIR 67/548/EEC, fluazinam should be classified as irritating to respiratory system (Hazard symbol Xi, risk phrase R37).

4.3.3 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam should be classified as irritating to respiratory system (Hazard symbol Xi, risk phrase R37), although at the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 29), fluazinam was not classified.

According to Regulation EC 1272/2008, fluazinam should be classified for Specific target organ toxicity –single exposure: signal word "Warning", hazard category 3, hazard statement H335 (May cause respiratory irritation).

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

In the first acute inhalation study (*Tobeta, 1988*), all animals showed reduced spontaneous movement, moist fur, nasal blot, cloudy eyeballs, decreased respiratory rate and gasping or abnormal breathing sound during exposure. Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered to be mostly due to respiratory failure.

In the second study (*Kirkpatrick, 2006*), significant clinical observations immediately following exposure included rales, closure of the eyes and red material around the eyes, nose and mouth. Clinical observations during the 14 days observation period consisted of rales, decreased defecation and urination, and red material around the eyes, nose and mouth. All surviving animals were considered normal by study day 6, and surpassed their initial body weight by study day 14.

Macroscopic finding noted for one male that died, was dark red discoloration of the lungs.

In both studies, necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities.

Therefore, according to the CLP Regulation, classification for Specific target organ toxicity – single exposure, STOT SE 3 (H335) is required. According to Directive 67/548/EEC, fluazinam should be classified as irritating to the respiratory system (Xi; R37).

Comments received during public consultation

- Three MSCAs did not support the classification with STOT SE.
- One MSCA supported the classification considering that transient effects were observed in the Kirkpatrick (2006) study.

RAC assessment - comparison with the classification criteria and justification

The CLP Regulation explains that classification for Specific Target Organ Toxicity – Single Exposure (STOT SE) should be considered where there is clear evidence of toxicity to a specific organ, especially when it is observed in the absence of lethality.

Comparison with the criteria:

In the Tobeta (1988), Kirkpatrick (2006) and Griffiths (2009) studies, mortality related to fluazinam exposure is observed and that is the reason why STOT SE 3 does not seem justified.

Conclusion:

When comparing the available data with the classification criteria, RAC concluded that classification of fluazinam as STOT SE 3 (H335) according to the CLP Regulation and as Xi; R37 according to Directive 67/548/EEC was not justified.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Dermal irritation study	Rabbit (NZW): mildly irritating	Slight to well defined erythema, no edema	*Shults, 1992*

4.4.1.1 Non-human information

Primary dermal irritation study in albino rabbits:

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

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

GLP: yes

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

Findings:
Clinical signs and mortality: No animals exhibited signs of systemic toxicity and no death occurred during the study. Slight to well defined erythema was observed in all 6 rabbits at the 30 and 60 minute interval and in 5 rabbits at the 24 and 48 hour intervals. On day 4, erythema was observed in 4, on day 5 in 3 animals and persisted till day 11 in 2 animals and in one rabbit till day 12. No edema was observed in any of the rabbits during the study. The primary irritation index for erythema was calculated to be 0.9.

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

Animal	Erythema													
	Min	Hour			Days									
	30-60	24	48	72	4	5	6	7	8	9	10	11	12	13
M1	1	1	1	1	1	1	1	1	1	1	1	1	0	0
M2	1	1	1	0	0	0	0	0	0	0	0	0	0	0

M3	1	0	0	0	0	0	0	0	0	0	0	0	0	0
F1	1	1	1	2	2	2	2	2	2	1	1	1	1	0
F2	1	1	1	1	1	1	0	0	0	0	0	0	0	0
F3	1	1	1	1	1	0	0	0	0	0	0	0	0	0
Mean	1.0	0.8	0.8	0.8	0.8	0.7	0.5	0.5	0.5	0.3	0.3	0.3	0.2	0.0

M = Male rabbit, F = Female rabbit

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

4.4.1.2 Human information

No data

4.4.1.3 Summary and discussion of skin irritation

Fluazinam is mildly irritating to the skin.

Repeated dermal administration of fluazinam at concentrations of 10, 100 and 1000 mg/kg bw to rats for 3 weeks revealed effects to the skin (acanthosis and dermatitis) compared to controls. So at the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 29), it was decided to classify fluazinam additionally as irritating to skin (hazard symbol Xi, risk phrase R38), based on macroscopic and microscopic changes in treated skin (acanthosis, dermatitis, scabs and ulceration).

4.4.1.4 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as irritating to skin (Hazard symbol Xi, risk phrase R38) and acute hazard category 2 for skin irritation and labeled with signal word "Warning" and hazard statement H315 (Causes skin irritation), respectively based on macroscopic and microscopic changes in treated skin (acanthosis, dermatitis, scabs and ulceration).

4.4.1.5 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as irritating to skin (Hazard symbol Xi, risk phrase R38).

According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 2 for skin irritation and labeled with signal word "Warning" and hazard statement H315 (Causes skin irritation).

RAC evaluation of skin irritation

Summary of the Dossier submitter's proposal

According to the Draize criteria, fluazinam is mildly irritating to the skin in the study of Shults (1992). The primary index for erythema was calculated to be 0.9 which does not justify any classification.

No erythema and no oedema were observed in any of the rabbits in the study of Makhteshim Chemical Works Ltd. (Leuschner, 2006). The differences in toxicological properties of technical fluazinam evaluated for Annex I inclusion and Makhteshim's technical fluazinam are potentially due to the presence of the toxicologically relevant Impurity 5-chloro-N-(3-chloro-5-trifluoromethyl-2-pyridyl)-α,α,α-trifluoro-4,6-dinitro-o-toluidine in the technical material of the basic submitter, which is absent from Makhteshim's technical material (see EFSA Scientific review 2008, 137).

Repeated dermal administration of fluazinam (Cummins, 1985) at concentrations of 10, 100 and 1000 mg/kg bw to rats for 3 weeks revealed effects to the skin (acanthosis and dermatitis) compared to controls.

Hence, at the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 29), it was decided to classify fluazinam additionally as irritating to skin (Xi; R38), based on macroscopic and microscopic changes in treated skin (acanthosis, dermatitis, scabs and ulceration).

According to the CLP Regulation fluazinam should be classified as Skin Irrit. 2 (H315) and according to Directive 67/548/EEC as Xi; R38, based on macroscopic and microscopic changes in treated skin (acanthosis, dermatitis, scabs and ulceration).

Comments received during public consultation

- Two MSCAs agreed with the proposal of the dossier submitter.
- Three MSCAs disagreed.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

The results of the study conducted according to OECD or US EPA for assessing skin irritating do not justify a classification.

When considering the 3-week study in rat (Cummins, 1985) in which acanthosis, dermatitis scabs and ulceration was seen, classification as Skin Irritant, category 2 could be justified. The criteria for classification are based on "inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia and scaling."

Some RAC members expressed that although it is allowed according to the CLP Regulation, it is unusual to use a repeated dermal toxicity study for Skin irritation classification when irritation studies are available.

Conclusion:

In view of the RAC members' comments and the irritation study conducted by Cumming and Chevallier (according to US EPA and OECD guidelines) as well as the results of the Shultz (1992) study, RAC concluded that fluazinam should not be classified as a skin irritant, neither according to the CLP Regulation nor to Directive 67/548/EEC.

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation study	Rabbit (NZW): severely irritating	Corneal, iridal and conjunctival effects persisted partly through day 21 of the study.	*Shults, 1992*

4.4.2.1 Non-human information

Primary eye irritation study in albino rabbits:

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

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

GLP: yes

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

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

Iridal effects were observed in 4 rabbits and persisted in one animal till termination on day 21. Conjunctival irritation was observed in all six rabbits at the 1 hour interval and persisted in one animal till day 21.

Individual eye irritation scores

Rabbit No.	Time after treatment	Corneal opacity	Iridial inflammation	Conjunctival redness	Conjunctival chemosis
	1 hour	0	0	2	2

202727	24 hours 48 hours 72 hours	2 2 2	1 1 1	3 3 3	4 3 2
	Mean 24 – 72 h	2	1	3	3
202728	1 hour	0	0	1	1
	24 hours 48 hours 72 hours	2 3 3	1 1 1	3 3 3	3 3 2
	Mean 24 – 72 h	2.6	1	3	2.6
202729	1 hour	0	0	1	1
	24 hours 48 hours 72 hours	2 1 0	0 0 0	3 2 1	3 1 1
	Mean 24 – 72 h	1	0	2	1.6
202730	1 hour	0	0	2	1
	24 hours 48 hours 72 hours	1 1 1	1 0 0	3 2 1	3 1 1
	Mean 24 – 72 h	1	0.3	2	1.6
202731	1 hour	0	0	2	1
	24 hours 48 hours 72 hours	1 1 1	1 0 0	3 3 2	3 2 1
	Mean 24 – 72 h	1	0.3	2.6	2
202732	1 hour	0	0	2	2
	24 hours 48 hours 72 hours	1 1 1	0 0 0	3 3 2	3 2 1
	Mean 24 – 72 h	1	0	2.6	2

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

4.4.2.2 Human information

No data.

4.4.2.3 Summary and discussion of eye irritation

Significant corneal epithelial effects involving up to approximately 25 % of the corneal surface in 3 rabbits at 72 hours were observed which persisted in 2 animals through day 7 of the study.

Iridal effects were observed in 4 rabbits and persisted in one animal till termination on day 21. Conjunctival irritation was observed in all six rabbits at the 1 hour interval and persisted in one animal till day 21. So Fluazinam is severely irritating to the eyes of New Zealand White rabbits.

4.4.2.4 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as severely irritating to the eyes (Risk of serious damage to eyes (Hazard symbol Xi, risk phrase R41) and hazard category 1 for eye damage and labeled with the signal word “Danger” and hazard statement H318 (Causes serious eye damage), respectively since corneal, iridal and conjunctival effects which persisted partly through day 21 of the study are reported.

4.4.2.5 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as severely irritating to the eyes (Risk of serious damage to eyes. Hazard symbol Xi, risk phrase R41).

According to Regulation EC 1272/2008, fluazinam should be classified in hazard category 1 for eye damage and labeled with the signal word “Danger” and hazard statement H318 (Causes serious eye damage).

RAC evaluation of eye corrosion / irritation

Summary of the Dossier submitter's proposal

In the study by Shults (1992), significant corneal epithelial effects involving up to approximately 25 % of the corneal surface in 3 rabbits at 72 hours were observed. The effect persisted through day 7 of the study in 2 rabbits.

Iridal effects were observed in four rabbits and persisted in one animal until termination of the study on day 21. Conjunctival irritation was observed in all six rabbits at the 1 hour interval and persisted in one animal until day 21. Hence, fluazinam has been shown to be severely irritating to the eyes of New Zealand White rabbits.

When comparing the criteria for classification and labelling according to Directive 67/548/EEC and the CLP Regulation with the effects seen in the eye irritation study by Leuschner (2006), fluazinam is not considered irritating to the eyes.

Considering the criteria for classification and labelling according to Directive 67/548/EEC and to the CLP Regulation, fluazinam has to be classified as severely irritating to the eyes (Xi; R41, Risk of serious damage to eyes) and Eye Dam. 1 (H318), respectively, since corneal, iridal and conjunctival effects which persisted partly through day 21 of the study are reported.

Comments received during public consultation

– Four MSCAs agreed with the dossier submitter's proposal.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

The classification is justified considering that "at least in one animal effects on cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days" are observed.

Conclusion:

When comparing the available data with the classification criteria, RAC concluded that classification as Eye Dam. 1 (H318) according to the CLP Regulation (Xi; R41 according to Directive 67/548/EEC) is justified.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

Please refer to 4.3: Specific target organ toxicity – single exposure (STOT SE).

4.4.3.2 Human information

No data.

4.4.3.3 Summary and discussion of respiratory tract irritation

Please refer to 4.3: Specific target organ toxicity – single exposure (STOT SE).

4.4.3.4 Comparison with criteria

In the first acute inhalative study (*Tobeta, 1988*) signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed at necropsy. Deaths were considered mostly due to respiratory failure. In the second study (*Kirkpatrick, 2006*) macroscopic finding noted for 1 male that died was dark red discoloration of the lungs. In both studies, necropsy of the surviving animals at the end of the 14-day observation period showed no abnormalities. Therefore, according to Regulation EC 1272/2008, classification for STOT SE Specific target organ toxicity –single exposure: Cat. 3, H335 is required. According to DIR 67/548/EEC, fluazinam should be classified as irritating to respiratory system (Hazard symbol Xi, risk phrase R37).

4.4.3.5 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam should be classified as irritating to respiratory system (Hazard symbol Xi, risk phrase R37), although at the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 29), fluazinam was not classified.

According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 3 for specific target organ toxicity after single exposure and labeled with signal word "Warning" and hazard statement H335.

4.5 Corrosivity

Based on the data from the skin irritation study, it can be concluded that fluazinam is not corrosive.

RAC evaluation of corrosivity

Summary of the Dossier Submitter's proposal

Based on the data from the skin irritation study, it can be concluded that fluazinam is not corrosive.

Comments received during public consultation

– One MSCA supported non-classification.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

Fluazinam does not meet the classification criteria according to the CLP Regulation (or Directive 67/548/EEC) since "A corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to 4 hour….".

Conclusion:

Classification for corrosivity is not justified.

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

Delayed contact hypersensitivity study in guinea-pigs:

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

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

GLP: yes

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

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

Dermal responses to primary induction were assessed 24 and 48 hours after administration.

Topical induction (for 48 hours under occlusive dressing at the injection test sites) was carried out on day 8 using a concentration of 0.4 ml 70 % (w/v) fluazinam in paraffin oil. Paraffin oil was used in replacement of fluazinam for the control group. Dermal responses to secondary induction were assessed 24 and 48 hours after removal of the occlusive dressing.

On day 22 the challenge phase was performed in the treated group and in the control group by applying 0.2 ml 70 % (w/v) solution of fluazinam in paraffin oil dermally under occlusive dressing for 24 hours on the right flank (50 x 50 mm area) while the left flank received the vehicle only. The dressings were removed 24 hours later and skin reactions were quantified 4, 24 and 48 hours thereafter macroscopically.

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

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

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

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

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

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

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

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

GLP: yes

Material and Methods:
Technical fluazinam (batch no. 5903-2 and 8412-20; purity 95.3 %) and purified fluazinam (batch no. 8505-1; purity 99.7 %) were used in this study.
Induction phase: 20 male guinea pigs (strain: Dunkin Hartley; source: Animal Breeding Unit, Imperial Chemical Industries PLC, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, UK), were treated topically with 0.4 ml of a 50 % w/v solution of fluazinam technical (batch no. 5903-2) in 0.5 % polysorbate 80. 10 male guinea pigs of the same strain served as controls and received 0.5 % polysorbate 80 only. Patches were applied onto the shaved left shoulder (50 mm x 50 mm) of the animals and removed after approximately 6 hours. These treatments were performed once a week, for three consecutive weeks. Following each induction, test sites were scored for dermal irritation 24 hours after removal of each patch and before application of each subsequent patch. Following the final induction application, animals were left untreated for a period of 14 days (rest phase). The concentration used for the treatment in this study was based on the results of a preliminary screening study and was the highest concentration which did not cause any irritation following a single application. Data of a positive control group are not reported.
For the challenge phase, flanks of the animals were shaved (150 mm x 50 mm). An occlusive dressing was prepared which consisted of 2 lint pads stitched to a piece of rubber sheeting. One lint pad (10 mm x 20 mm) containing 0.2 ml of a 50 % w/v suspension of fluazinam technical (batch no. 5903-2) in 0.5 % polysorbate 80 was applied on the right flank and the second lint pad containing 0.2 ml of a 50 % w/v suspension of purified fluazinam in 0.5 % polysorbate 80 was applied on the left flank. Test sides were occluded for 6 hours. At approximately 24 hours after patch removal, test sites were graded for dermal irritation (24-hour scoring period) and additionally after further 24 hours (48-hour scoring period).
14 days after the initial challenge, test animals were given a further topical induction of a 50 % w/v suspension of fluazinam technical (batch no. 5903-2). Seven days after the second induction animals were rechallenged using 50 % (w/v) preparations of both technical (batch no. 8412-20) and

purified fluazinam in 0.5 % polysorbate 80. Both flanks were clipped free of hair and fluazinam was applied to different sites than those used for the initial challenge. A fresh group of ten male control animals was used for the rechallenge.

Findings:
Signs of moderate skin irritation (erythema, desquamation, thickening, edema and scabbing) were seen after the second and third inductions. Nine of 20 test animals and one of 10 controls had scattered mild or moderate and diffuse redness after challenge with the technical material. The net percentage response was 35% and, therefore, a 50% preparation of technical fluazinam elicited a moderate sensitization response in previously induced guinea pigs.
Three of 20 test animals and one of 10 controls had scattered mild redness after rechallenge with purified fluazinam. The net percentage response was 5% and, therefore, a 50% preparation of purified fluazinam elicited a weak sensitization response in previously induced guinea pigs.

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

Table 14: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Dermal sensitization M & K-test	Guinea pig (Dunkin Hartley): Sensitizing	delayed contact hypersensitivity	*Cummins, 1984*
Dermal sensitization Buehler –test	Guinea pig (Dunkin Hartley): Sensitizing	moderate sensitization response	*Pritchard, 1986*

4.6.1.2 Human information

No data.

4.6.1.3 Summary and discussion of skin sensitisation

In the Magnusson and Kligman dermal maximization study and in the Buehler-Test fluazinam caused evidence of delayed contact hypersensitivity in guinea pigs.

4.6.1.4 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as a sensitizer (hazard symbol Xi, risk phrase R43) and acute hazard category 1 for dermal sensitization and labeled with signal word “Warning” and hazard statement H317 (May cause an allergic skin reaction), respectively since in both skin sensitization studies (Magnusson and Kligman, Buehler) delayed contact hypersensitivity in guinea pigs was observed.

4.6.1.5 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as a sensitizer (hazard symbol Xi, risk phrase R43).

According to Regulation EC 1272/2008, fluazinam should be classified in acute hazard category 1 for dermal sensitization and labeled with signal word "Warning" and hazard statement H317 (May cause an allergic skin reaction).

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

In the Magnusson and Kligman dermal maximization study by Cummins (1984), and in the Buehler test by Pritchard (1986), fluazinam caused evidence of delayed contact hypersensitivity (redness) in guinea pigs. In the Magnusson and Kligman dermal maximization study by Chevalier (2006), none of the test group animals showed a dermal reaction after challenge.

Considering the criteria for classification and labelling, and Directive 67/548/EEC, fluazinam has to be classified as Skin Sens. 1A (H317) according to the CLP Regulation (R43 according to Directive 67/548/EEC) since in skin sensitization studies (Buehler) delayed contact hypersensitivity (redness) in guinea pigs was observed in 35 % of the tested animals.

Comments received during public consultation

Four MSCAs made comments.

- Three agreed with the dossier submitter's proposal.
- One MSCA requested more information about the tests.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

The intradermal induction was 0.2 % and redness was observed in > 15 % of the tested animals which justifies a classification as Skin Sens. 1A (H317) according to the CLP Regulation (R43 according to Directive 67/548/EEC).

Conclusion:

When comparing the available data with the classification criteria, RAC concluded that classification as Skin Sens. 1A (H317) according to the CLP Regulation (R43 according to Directive 67/548/EEC) is justified.

4.6.2 Respiratory sensitisation

Based on the data from the acute inhalative studies, it can be concluded that fluazinam is not a respiratory sensitizer.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

Due to lack of data, no classification for fluazinam for respiratory sensitisation is proposed.

Comments received during public consultation

Five comments were received during public consultation.

Four MSCAs made comments.

- Three MSCAs agreed with the non classification.
- One MSCA found a publication which discusses occupational asthma caused by sensitisation to powdered fungicides fluazinam and chlorotalonil. Furthermore, because this substance is already classified as a skin sensitizer it is important to consider all the available data.
- One manufacturer agreed with the proposal of non classification.

RAC assessment - comparison with the classification criteria and justification

Conclusion:

Based on a lack of data, no classification is proposed for fluazinam for this hazard class.

4.7 Repeated dose toxicity

Table 15: Summary table of relevant repeated dose toxicity studies

• Method	• Dose levels	• NOAEL	• Remarks (Relevant effects at the LOAEL)	• Reference
• CD rats 4 weeks oral	• 0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.26, 5.21, 26.1 and 305.4 mg/kg bw)	• 5.21 mg/kg bw/d	• -reduced food consumption and body weight gain -clinical chemical findings -higher absolute and relative liver weights	• *Broadmeadow A. et al; 1983*
• CD rats 13 weeks oral	• 0, 2, 10, 50 and 500 ppm/diet (equivalent to 0, 0.16, 0.82, 4.1 and 41 mg/kg bw)	• 4.1 mg/kg bw/d	• hematological findings -higher relative liver weights -histopathological changes in the liver	• *Broadmeadow A. et al; 1984*
• CD rats 21 days dermal	• 0, 10,100 and 1000 mg/kg bw)	• Cannot be determined	• -clinical chemical findings -histopathological changes in the skin	• *Cummins H. A. et al; 1985*
• CD-1 mice 4 weeks oral	• 0, 10, 50, 250 and 3000 ppm/diet (equivalent to 0, 1.6, 7.9, 39.5 and 455 mg/kg bw)	• 7.9 mg/kg bw/d	• reduced food consumption and body weight gain -clinical chemical findings -higher absolute and relative kidney weights	• *Amyes S. J. et al; 1983*
• Beagle dogs 4 weeks oral	• 0, 1, 5, 25 and 150 mg/kg bw, gelatine capsules)	• 5 mg/kg bw/d	• -grey pigmentation of the tapetal fundus of the retina -higher relative liver weights	• *Broadmeadow A. et al; 1984*
• Beagle dogs 13 weeks oral	• 0, 1,10 and 100 mg/kg bw, gelatine capsules)	• 10mg/kg bw/d	• reduced food consumption and body weight gain -grey pigmentation of the tapetal fundus of the retina -clinical chemical findings	• *Broadmeadow A. et al; 1985*

			-higher absolute and relative liver weights -histopathological changes in the liver	
• Beagle dogs 52 weeks oral	• 0, 1, 10 and 50 mg/kg bw, gelatine capsules)	• 1mg/kg bw/d	• hematological and clinical chemical findings -bone marrow smears: myeloid to erythroid ratio increased -higher absolute and relative liver weights -histopathological changes in the stomach	• *Broadmeadow A. et al; 1987*

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Subacute and subchronic administration of fluazinam to rats, mice and dogs caused reduced food consumption and body weight gain. Changes of hematological parameters such as lower haemoglobin concentrations, lower erythrocyte counts and lower platelet counts were also observed. Clinical chemistry parameters showed low ALT activity, higher cholesterol, phospholipid and glucose concentrations. Higher absolute and relative liver weights and histopathological changes in the liver such as periacinar hepatocytic hypertrophy were observed in all species. In mice and dogs, vacuolation of white matter in brain and spinal cord was observed at high dose levels (mice at a dose level of 600 mg/kg bw/d for 4 weeks, dogs at a dose level of 50 mg/kg bw/d for 52 weeks). The changes in brain and spinal cord were not due to fluazinam itself, but rather to a manufactory impurity, called Impurity-5 and were found to be reversible.
High dosed dogs of the 4- and 13-week oral toxicity studies (150 and 100 mg/kg bw/d resp.) showed retinal hyperreflection and grey pigmentation of the tapetal fundus of the retina. At histopathologic examination, a dystrophy of the pigment epithelium of the retina was observed in the majority of dogs, including controls. The toxicological significance of the ophthalmic observations and the possible interrelationships between these and the retinal findings observed histopathologically were unknown. Oral administration of 200/150 mg/kg bw/d fluazinam to beagle dogs for 11 weeks revealed ERG-abnormalities which can be accounted for by functional changes in the pigment epithelium of the retina. The results show recovery of response amplitude after withdrawal of fluazinam, but it is not possible to say if recovery would be complete.

4.7.1.2 Repeated dose toxicity: inhalation

No data available.

4.7.1.3 Repeated dose toxicity: dermal

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

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

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

Material and Methods:

Groups of 10 rats/sex (strain: Sprague-Dawley (CD); source: Charles River (U.K.) Limited) received doses of 10, 100 and 1000 mg/kg bw fluazinam (batch no. 8303-2; purity 98.5 %) by occluded application to the shaven skin for 6 hours per day for 21 days. An additional group of 10 males and 10 females received the vehicle only, 0.5% methyl cellulose, to serve as controls. Animals were observed for clinical signs and mortality 4 times per day and dermal reactions were assessed daily. Body weight and food consumption were recorded weekly. Hematology and blood chemistry were analyzed on day 20. All animals were necropsied and the weights of selected organs (adrenals, brain, kidneys, liver, ovaries, testes) were recorded. Histopathological examinations were performed on heart, kidneys, liver, lungs, ovaries, skin, stomach and testes and any tissue showing macroscopic abnormality.

Findings:

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

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

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

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

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

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

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

Conclusion:

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

At the PRAPeR Experts' Meeting on mammalian toxicology (PRAPeR 29), it was decided to classify fluazinam additionally as irritating to skin (hazard symbol Xi, risk phrase R38), based on macroscopic and microscopic changes in treated skin (acanthosis, dermatitis, scabs and ulceration).

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

No data available.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Subchronic toxicity tests were conducted in rats, mice and dogs. The main target organ was the liver. White matter vacuolation in the brain in mice and dogs is not due to fluazinam itself, but rather to a manufactory impurity, called Impurity-5. All vacuolation effects were found to be reversible. There is a non-linear dose-response for the production of white matter vacuolation with a threshold, below which no white matter vacuolation occurs, at approximately 0.1 mg/kg bw/d of Impurity-5.

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD

The available information indicates that classification for repeat dose toxicity is not warranted.

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD

Considering the criteria for classification and labelling according to Directive 67/548/EEC and REG 1272/2008, no classification for Fluazinam considering repeated dose toxicity is considered necessary.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD

The available information indicates that classification for repeated dose toxicity is not warranted.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

Under the CLP Regulation, the harmful (Xn) classification cut-off values (guidance values) are higher: 100 mg/kg/day for a 90-day study and 300 mg/kg/day for a 28-day study in rats. However, as there were no serious effects below either of these guidance values in all three species investigated, classification for STOT- RE under the CLP Regulation is not warranted.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

Subchronic toxicity tests were conducted in rats, mice and dogs. The main target organ was the liver. White matter vacuolation in the brain in mice and dogs is not considered to be due to fluazinam itself, but rather to a manufactory impurity, called Impurity 5. All vacuolation effects were found to be reversible. There is a non-linear dose-response for the production of white matter vacuolation with a threshold, below which no white matter vacuolation occurs, at approximately 0.1 mg/kg bw/d of Impurity 5.

Specific target organ toxicity – repeated exposure (STOT RE) (CLP Regulation): The cut-off values (guidance values) for STOT RE classification according to the CLP Regulation are higher than the ones for the corresponding classification ("harmful"; Xn) according to Directive 67/548/EEC: 100 mg/kg/day for a 90-day study and 300 mg/kg/day for a 28-day study in rats. However, as there were no serious effects below either of these guidance values in all three species investigated, classification for STOT RE under the CLP Regulation is not warranted.

Considering the criteria for classification and labelling according to Directive 67/548/EEC and the CLP Regulation, no classification for fluazinam for repeated dose toxicity is considered necessary. The available information indicates that classification for repeated dose toxicity is not warranted.

Comments received during public consultation

Three MSCAs made comments.

- One MSCA agreed with dossier submitter's classification proposal.
- The others requested more information, mentioning the difficulty to assess the relevance of the impurity for the classification and recommend using the two carcinogenicity studies in rats and mice.

RAC assessment - comparison with the classification criteria and justification

The dermal repeated toxicity studies shows a clear effect on liver by the oral and dermal exposure route in three species, and on skin by dermal route which could support classification for STOT RE. The effects observed are absolute and relative liver weight increase as well as histopathological changes. No information on their statistical significance is provided. These effects are not considered as serious.

No information is given on the doses or concentration at which the effects are observed.

In the oral carcinogenicity studies in rats the non-neoplastic effects on liver and testes were manifested at 100 ppm (< 5 mg/kg), which could justify a classification for STOT RE category 1.

Conclusion:

Based on effects, which are not considered as serious, and the absence of sufficient information (protocol, doses and findings as reversibility) to support the data, no classification for STOT RE (Specific target organ toxicity, repeated exposure) is proposed.

4.9 Germ cell mutagenicity (Mutagenicity)

The mutagenicity of fluazinam has been adequately investigated *in vitro* and *in vivo*.

Table 16: Summary table of relevant in vitro and in vivo mutagenicity studies

Type of study	Test system	Dose range	Results	Reference
In vitro-studies				
Bacterial mutation assay	S. typhimurium (TA1535, TA1537, TA98 and TA100) and E. coli WP2uvrA/pKM 101 (CM891)	0.005, 0.015, 0.050, 0.15, 0.5, 1.5, 5, 15, 50, 150, 500, 1500 and 5000 µg/plate	Negative	*Kitching J.;2000*
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0625 - 2 µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 - 500 µg/plate (with S-9 mix)	Negative	*Ohtsuka M.; 1988*
Bacterial reverse mutation test	*S. typhimurium* (TA100, TA1535, TA98 and TA1537) *E. coli* (WP2 uvr A)	0.0313 - 1µg/plate (without S-9 mix), 3.13 - 100 µg/plate (with S-9 mix) 15.6 - 250 µg/plate (without S-9 mix), 31.3 - 500 µg/plate ((with S-9 mix)	Negative	*Ohtsuka M.; 1989*
Mammalian cell mutation assay	mouse lymphoma L5178Y cells	First test: 0.05 - 5 µg/ml (without S-9 mix); 0.5 - 20 µg/ml (with S-9 mix) Second test: 0.005 - 0.5 µg/ml (without S-9 mix); 0.5 - 10 µg/ml (with S-9 mix)	Negative	*Ransome S.; 2000*
Chromosomal aberration test	CHL	1 - 4 µg/ml (with S-9 mix); 2.375 - 9.5 µg/ml (without S-9 mix)	Negative	*Kajiwara Y.; 1988*
DNA repair test	bacillus subtilis	0.003 - 0.3 µg/disk (without S-9 mix), 0.3 – 30 µg/disk (with S-9 mix)	negative	*Ohtsuka M.; 1988*
In vivo-studies				
Micronucleus test	mouse bone marrow	single oral doses of 0, 500, 1000 and 2000 mg/kg bw	negative	*Matsumoto K.; 1999*

4.9.1 Non-human information

4.9.1.1 In vitro data

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

4.9.1.2 In vivo data

In the *in vivo micronucleus test* no induction of micronuclei by fluazinam in mouse bone marrow cells could be observed

4.9.2 Human information

No data available.

4.9.3 Other relevant information

No data available.

4.9.4 Summary and discussion of mutagenicity

Mutagenicity assays performed *in vitro* included gene mutation tests in bacteria (*S. typhimurium and E.coli*) and in mammalian cells (*mouse lymphoma*), a chromosomal aberration test in mammalian cells (Chinese hamster lung fibroblasts) and a DNA repair test in bacteria (Bacillus subtilis). All the results were negative, showing that fluazinam has no genotoxic potential *in vitro*. In the *in vivo* micronucleus test, no induction of micronuclei could be observed, therefore no evidence of genotoxic potential *in vivo* has been shown.

4.9.5 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, no classification for Fluazinam considering mutagenic effects is considered necessary.

4.9.6 Conclusions on classification and labelling

Data indicate that fluazinam is not mutagenic *in vitro* or *in vivo* and does not meet the criteria for classification as a mutagen.

RAC evaluation of mutagenicity

Summary of the Dossier Submitter's proposal

Mutagenicity assays performed *in vitro* included gene mutation tests in bacteria (*S. typhimurium and E.coli*) and in mammalian cells (*mouse lymphoma*), a chromosomal aberration test in mammalian cells (Chinese hamster lung fibroblasts) and a DNA repair test in bacteria (Bacillus subtilis). All the results were negative, showing that fluazinam has no genotoxic potential *in vitro*.

In the *in vivo* micronucleus test, no induction of micronuclei could be observed, therefore no evidence of genotoxic potential *in vivo* has been shown.

Considering the criteria for classification and labelling according to Directive 67/548/EEC and the CLP Regulation, no classification of fluazinam for mutagenic effects was considered necessary by the dossier submitter.

Comments received during public consultation

– One MSCA agreed with the proposal not to classify fluazinam as mutagenic.

– One MSCA requested study summaries and that was provided.

RAC assessment - comparison with the classification criteria and justification

Conclusion:

The available data indicates that fluazinam is not mutagenic *in vitro* or *in vivo* and does not meet the criteria for classification for mutagenicity.

4.10 Carcinogenicity

Table 17: Summary table of relevant carcinogenicity studies

Study; Reference	Dose levels	NOAEL	Main effects/target organs
Sprague-Dawley rats 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.04, 0.38, 3.82 and 40 mg/kg bw males, 0, 0.05, 0.47, 4.87 and 53 mg/kg bw females)	10 ppm (0.38 mg/kg bw males, 0.47 mg/kg bw females)	-hematological and clinical chemical findings -higher liver and thyroid weights -histopathological changes in liver, pancreas, lungs and testes
Sprague-Dawley rats 104 weeks oral *Chambers P. R. et al; 1993*	0, 25, 50 and 100 ppm/diet (equivalent to 0, 1.0, 1.9 and 3.9 mg/kg bw males, 0, 1.2, 2.4 and 4.9 mg/kg bw females)	50 ppm (1.9 mg/kg bw males, 2.4 mg/kg bw females)	-higher liver, testes and epididymides weights -histopathological changes in liver, pancreas, lungs and testes
CD-1 mice 104 weeks oral *Mayfield R. et al; 1988*	0, 1, 10, 100 and 1000 ppm/diet (equivalent to 0, 0.12, 1.12, 10.72 and 107 mg/kg bw males, 0, 0.11, 1.16, 11.72 and 117 mg/kg bw females)	10 ppm (1.12 mg/kg bw males, 1.16 mg/kg bw females)	-higher liver weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord

Study; Reference	Dose levels	NOAEL	Main effects/target organs
CD-1 mice 104 weeks oral *Chambers P. R. et al; 1998*	0, 1000, 3000 and 7000 ppm/diet (equivalent to 0, 126, 377 and 964 mg/kg bw males, 0, 162, 453 and 1185 mg/kg bw females)	Cannot be determined	-higher liver, brain and adrenal weights -histopathological changes in liver, liver cell tumours -vacuolation of white matter in brain and spinal cord

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

In the two long term toxicity/carcinogenicity studies in rats, treatment-related non-neoplastic effects were manifest at 100 ppm especially in the liver and testes. No treatment-related effects were seen on the spontaneous tumor profile at any dose level. Taking the two long term toxicity/carcinogenicity studies in rats together, an overall NOAEL for fluazinam can be obtained at 50 ppm, equivalent to 1.9 mg/kg bw/d for males and 2.4 mg/kg bw/d for females.

In two carcinogenicity studies in mice, liver cell tumours (adenomas and carcinomas) were observed in a greater number of male mice after dietary administration of 1000, 3000 and 7000 ppm fluazinam, reaching statistical significance for adenomas at dose levels of 1000 (33 %) and 3000 ppm (40 %) only. The historical control data for liver tumours carried out at Huntingdon Research Centre Ltd. in the years 1981 – 1983 and 1991 – 1993 showed incidences of adenomas in the range of 3.8 to 34 %. Thus the incidence of liver tumours at 1000 and 3000 ppm were within or slightly above the range of the historical control data. However, hepatocellular adenomas in the highest dose group of 7000 ppm reached an incidence of 28% and were within the range of the historical controls.

A statistically significant increase of vacuolation of white matter in the brain and cervical spinal cord was observed in both sexes at dose levels of 1000 ppm fluazinam and above. The changes in brain and spinal cord were not due to fluazinam itself, but rather to a manufactory impurity, called Impurity-5 and were found to be reversible.
10 ppm, equivalent to 1.12 mg/kg TG/d for males and 1.16 mg/kg TG/d for females, were considered to be the NOAEL in carcinogenicity studies in mice.

4.10.1.2 Carcinogenicity: inhalation

No data available.

4.10.1.3 Carcinogenicity: dermal

No data available.

4.10.2 Human information

No data available.

4.10.3 Other relevant information

No data available.

4.10.4 Summary and discussion of carcinogenicity

In the two chronic/carcinogenicity studies with rats, the main changes were observed in the liver, pancreas, lung, lymph nodes and testes. No increased incidence of tumours was shown. The resulting overall NOAEL is 1.9 mg/kg bw/d.

In the two long term studies with mice, the NOAEL for general toxicity is 1.12 mg/kg bw/d based on effects in the liver. An increased incidence of liver cell tumours (adenomas and carcinomas) was observed in males at 107 mg/kg bw/d and above and was within the historical control range in the highest dose group.
In addition, vacuolation of white matter in the brain and cervical spinal cord was observed in both sexes at dose levels of 107 mg/kg bw/d and above. The changes in brain and spinal cord were not due to fluazinam itself, but rather to a manufactory impurity, called Impurity-5 and were found to be reversible.
The resulting overall NOAEL is 1.12 mg/kg TG/d.

4.10.5 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, no classification for Fluazinam considering carcinogenic effects is considered necessary.

4.10.6 Conclusions on classification and labelling

Data indicate that fluazinam does not meet the criteria of classification for carcinogenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

In the two long term toxicity/carcinogenicity studies in rats (Mayfield R *et al*., 1988 and Chambers P.R *et al*., 1993), treatment-related non-neoplastic effects were manifest at 100 ppm, especially in the liver and testes. No treatment-related effects were seen on the spontaneous tumour profile at any dose level. Taking the two long term toxicity/carcinogenicity studies in rats together, an overall NOAEL for fluazinam can be obtained at 50 ppm, equivalent to 1.9 mg/kg bw/d for males and 2.4 mg/kg bw/d for females.

In two carcinogenicity studies in mice (Mayfield R *et al*., 1988 and Chambers P.R *et al*., 1998), liver cell tumours (adenomas and carcinomas) were observed in a greater number of male mice after dietary administration of 1000, 3000 and 7000 ppm fluazinam, reaching statistical significance for adenomas at dose levels of 1000 (33 %) and 3000 ppm (40 %) only. The historical control data for liver tumours carried out at Huntingdon Research Centre Ltd. in the years 1981 – 1983 and 1991 – 1993 showed incidences of adenomas in the range of 3.8 to 34 %. Thus the incidence of liver tumours at 1000 and 3000 ppm were within or slightly above the range of the historical control data. However, hepatocellular adenomas in the highest dose group of 7000 ppm reached an incidence of 28 % and were within the range of the historical controls.

A statistically significant increase of vacuolation of white matter in the brain and cervical spinal cord was observed in both sexes at dose levels of 1000 ppm fluazinam and above. The changes in brain and spinal cord were not due to fluazinam itself, but rather to a manufactory impurity, called Impurity 5 and were found to be reversible.

10 ppm, equivalent to 1.12 mg/kg TG/d for males and 1.16 mg/kg TG/d for females, were considered to be the NOAEL in carcinogenicity studies in mice.

Considering the criteria for classification and labelling according to the CLP Regulation and Directive 67/548/EEC, no classification for fluazinam for carcinogenic effects was considered necessary by the dossier submitter.

Comments received during public consultation

Three Members State Competent Authorities (MSCAs) made comments:

- Two MSCAs requested more information for all tumour incidences.
- One MSCA agreed with the dossier submitter's proposal not to classify for carcinogenicity.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

Considering the criteria for classification and labelling according to the CLP Regulation and Directive 67/548/EEC, no classification for fluazinam for carcinogenic effects is considered necessary. In fact, in male mice, the observed liver tumours were within the historical control data and in rats no increased incidence of tumours was shown.

Conclusion:

The available data indicate that fluazinam does not meet the criteria for classification for carcinogenicity.

4.11 Toxicity for reproduction

Table 18: Summary table of relevant reproductive toxicity studies

Study Reference	Dose levels	NOAEL	Main effects/target organs
Two generation reproduction, rats *Tesh J. M. et al; 1987*	0, 20, 100 or 500 ppm, equivalent to 0, 1.5, 7.2 and 36.5 mg/kg bw/d males; 0, 1.7, 8.4 and 43 mg/kg bw/d females (average achieved intake during prepairing period)	Parental 20 ppm (1.5 mg/kg bw/d males, 1.7 mg/kg bw/d females) Reproductive 100 ppm (7.2 mg/kg bw/d males, 8.4 mg/kg bw/d females)	Parental: body weight and body weight gain ↓; relative liver weight ↑; histopathological liver changes Offsprings: gestation length ↑; conception rate, fertility index, implantation sites and litter sizes↓
Teratology in the rabbit *Tesh J. M. et al; 1985*	0, 0.3, 1 and 3 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 3 mg/kg bw/d Developmental NOAEL 1 mg/kg bw/d	Maternal: food consumption ↓ Developmental: ossification incomplete
Teratology in the rabbit *Tesh J. M. et al; 1988*	0, 2, 4, 7 and 12 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 4 mg/kg bw/d Developmental NOAEL 7 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓; histopathological liver changes Developmental: postimplantation loss ↑; placental and skeletal abnormalities ↑ (kinked tail tip, fused or incompletely ossified sternebrae, abnormalities of head bones)
Teratology in the rat *Willoughby C. R. et al; 1985*	0, 10, 50 and 250 mg/kg bw/d (oral application by gavage)	Maternal NOAEL 10 mg/kg bw/d Developmental NOAEL 10 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓ Developmental: fetal and placental weight ↓; ossification incomplete; gross morphological fetal abnormalities
Teratology in the rat *Beck M.; 2006*	0, 10, 50 and 300 mg/kg bw/d (oral application by gavage)	Maternal 10 mg/kg bw/d Developmental 10 mg/kg bw/d	Maternal: food consumption ↓; weight gain ↓; liver weights ↑ Developmental: postimplantation loss ↑; viable foetuses ↓; fetal weight ↓; renal papillae not developed; distended ureters; ossification incomplete

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Single and multi-generation studies in rats

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

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

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

The study is in compliance with GLP.

Material and method:

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

The average achieved intakes of fluazinam for the F_0 generation were equivalent to 0, 1.5, 5 and 26 mg/kg bw in males and 0, 1.7, 6.7 and 34 mg/kg bw in females. For the F_1 generation the average achieved intakes were 0, 1.9, 6 and 30 mg/kg bw in males and 0, 2.2, 7.5 and 40 mg/kg bw in females (lowest value of the range).

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

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

Parameters were calculated as follows:

Percentage mating: animals mating/animals paired X 100

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

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

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

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

Specified organs from all F_0 and F_1 parental animals (liver, ovaries, prostate with seminal vesicles, testes with epididymides and uterus) were weighed. Histopathological examinations were performed on these organs and on vagina, pituitary (animals of suspect fertility) and on all abnormalities from control and high dose animals of the F_0 and F_1 generation. Examination was extended to the livers of males from the lowest and intermediate dietary concentration groups.

Mammary tissue from any female which showed total litter loss was also examined microscopically.

Findings:

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

Mean group body weights of parental animals

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

	0 ppm	**20 ppm**	**100 ppm**	**500 ppm**
Day 1	324	324	315	301
Day 21	343	347	340	330
F_1 mean maternal body weight (g) during lactation				
	0 ppm	**20 ppm**	**100 ppm**	**500 ppm**
Day 1	314	313	315	281**
Day 21	338	334	329	302

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

Mating performance, pregnancy rate and gestation index of the F_0 generation were not adversely affected by treatment at any dose level. Gestation length was slightly increased in the high dose group. Implantation sites and mean litter sizes were within the laboratory background control ranges. In the F_1 generation, conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups. Numbers of implantation sites and mean litter sizes to day 4 post partum were slightly reduced for F_1 animals of the high dose group and marginally, but not statistically significant, lower in the intermediate group (100 ppm). In both generations, survival and lactation indices and sex ratios were unaffected by treatment. Birthweight of F_1 was similar in all groups but body weight gain during lactation period was reduced in the 500 ppm group. At birth, bodyweights of F_2 pups of the 500 ppm and 100 ppm groups were slightly increased compared to controls, whereas bodyweight gain of offspring to weaning was reduced at 500 ppm.

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

Mating performance, fertility and litter data (F0 generation, mean group values)

	0 ppm	20 ppm	100 ppm	500 ppm
Gestational length (days)	22.5	22.5	22.5	23
Conception rate (%)	96	100	96	100
Fertility index (%)	96	100	96	100
Implantation sites	15.0	15.5	16.0	14.3
Litter size total day 1	14.8	14.5	14.2	14.5
Litter size live day 1	13.2	14.2	14.4	12.4
Litter size live day 4	13.0	13.8	13.6	11.9
Litter size live day 21	7.8	7.9	7.8	7.4
Post implantation survival index (%)	88	92	91	88
Viability index (%)	94	98	95	87
Lactation index (%) day 7 p.p.	100	100	100	99
Lactation index (%) day 21 p.p.	99	100	99	97
mean pup weight (g) day 1 p.p.	6.3	6.1	6.2	6.1
mean pup weight (g) day 4 p.p. (before	9.0	8.3	8.3	8.5

cull) mean pup weight (g) day 21 p.p. (postcull)	53.5	51.7	52.0	48.4***
Pinna unfolding, completion (day p.p.)	3.3	3.4	3.5	3.1
Hair growth, completion (day p.p.)	3.0	3.3	3.3	3.3
Tooth eruption, completion (day p.p.)	10.5	11.2	10.8	10.7
Eye opening, completion (day p.p.)	14.7	14.7	14.5	13.8**

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

Mating performance, fertility and litter data (F1 generation, mean group values)

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

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

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

only). When organ weights were related to bodyweight, however, the only statistically significant finding was an increase in liver weight in males receiving 500 ppm.

Absolute (g)/relative (%) organ weights of F0 and F1 parental animals (mean group values)

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

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

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

Conclusion:

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

The NOAEL for systemic toxicity was considered to be 20 ppm, equivalent to approximately 1.5 mg/kg bw/d for males and 1.7 mg/kg bw/d for females.
For reproductive parameters, the NOAEL was considered to be 100 ppm, 7.26 mg/kg bw/d for males and 8.43 mg/kg bw/d for females.

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Teratology study in the rabbit:

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

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

The study is in compliance with GLP.

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

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

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

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

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

Reproduction data for does treated with fluazinam (mean group values)

Dose (mg/kg/bw/d)	0	0.3	1	3

No. of mated females	24	20	20	20
Not pregnant	1	3	3	4
Mortality	4	0	1	1
Abortion	0	1	0	1
Total litter loss	0	0	1	0
Pregnant to term with live young	18	16	15	14

Percentage of mean fetal observations at skeletal examination (number of litters)

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

Conclusion:

Under the conditions of this study, a NOAEL for maternal toxicity of 3 mg/kg bw/d can be obtained, based on reduced food intake in the high dose group. The NOAEL for fetal toxicity can be established at 1 mg/kg bw/d, based on incomplete ossification in the high dose group.
There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d).

Teratology study in the rabbit:

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

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

Material and method:
4 groups of 16 to 17 mated female New Zealand White rabbits (source: Ranch Rabbits, Crawley Down, Sussex, England), approximately 21 to 40 weeks old at commencement of the study, received oral doses (gavage) containing 2, 4, 7 and 12 mg/kg bw fluazinam (batch Lot 8412-20, purity 95.3 %) from day 6 to 19 of gestation. 18 animals served as controls, receiving the vehicle 1 % w/v aqueous methylcellulose mucilage by intubation. Diets were prepared daily; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Animals were checked daily for mortalities or signs of reaction. Food consumption was recorded for each animal during the following phases of the study: days 1 - 5, days 6 – 12, days 13 – 19, days 20 – 23 and days 24 - 28 post coitum. Body weights were recorded each day prior to dosing and mean values were calculated on days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. On day 29 post coitum, females were killed and the foetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. Liver and lungs were retained from all animals. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead foetuses recorded. Foetuses were removed, sexed, weighed and examined externally for gross abnormalities. All foetuses were dissected and examined internally. Placentae were weighed and examined for external abnormalities.

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

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

Mean maternal body weight (kg) during gestation

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

*: significantly different from control at $p<0.05$

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

Microscopic Findings in the liver (16 animals/dose group) of does

	Dose (mg/kg bw/day)				
Finding	**0**	**2**	**4**	**7**	**12**
Increased apoptosis	0	0	0	2	2
Necrosis of occasional single hepatocyte	0	0	0	2	4
Hepatocytes containing increased brown pigment	0	0	0	3	2
Foci of hepatocytic necrosis	0	0	0	0	2

	Dose (mg/kg bw/day)				
Finding	**0**	**2**	**4**	**7**	**12**
Occasional bile plugs within distended canaliculi	0	0	0	0	1
Centriacinar hypertrophy, slight	0	0	2	2	0
Panacinar hypertrophy, slight	0	0	1	3	2
Moderate	0	0	0	2	5
Marked	0	0	0	0	2

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

Reproduction data for female rabbits treated with fluazinam (mean group values)

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

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

Group mean litter data for female rabbits treated with fluazinam

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

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

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

Percentage of fetal observations at skeletal examination (number of litters)

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

+: Value above historical control high value

Conclusion:
Oral administration of fluazinam to pregnant rabbits during the period of organogenesis was associated with reduced maternal weight gain and food intake in the highest dose group of 12 mg/kg bw/d. Macroscopic and microscopic lung and liver changes reached statistical significance at a dose level of 7 mg/kg bw/d and above. So the maternal NOAEL can be considered at 4 mg/kg/day. Increased incidences of fetal abnormalities (placental abnormalities, some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were seen at the top dose. At all dose levels, increased incidences of preimplantation losses were observed, however, the values fell within the recorded background control range of the laboratory.
Postimplantation loss was increased at 4 mg/kg/day compared to concurrent controls, however, no increase was observed at the 7mg/kg/day dose level. As statistical significance was only reached at a dose level of 12 mg/kg bw/d, the NOAEL for fetal toxicity can be considered at 7 mg/kg bw/d.

Teratology study in the rat:

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

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

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

Findings:
14 animals in the high dose group (250 mg/kg bw/d fluazinam) showed urogenital staining during the dosing phase. In other respects, the general condition of the treated females was similar to that of the controls throughout the study. Mean food consumption of animals treated with 250 mg/kg bw/d fluazinam was reduced statistically significant during the early dosing period. In the 50 mg/kg bw/d group, food consumption was reduced during the early part of the dosing period too, but statistical significance was not reached. At 10 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.

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

Mean maternal body weight gains (g) during gestation

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

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

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

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

Group mean litter data for female rats treated with fluazinam

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

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

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

Incidences of facial/palatal clefts and/or incomplete ossification of palatine bones and diaphragmatic hernia in foetuses of high dose animals (250 mg/kg bw/d)

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

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

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

Percentage of fetal observations at skeletal examination (number of litters)

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

Incomplete ossification of pubic bones	10.7 (7)	15.7 (12)	12.2 (9)	22.5 (14)	0.0 – 16.0	0.0 – 3.1

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

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

A prenatal developmental toxicity study of technical fluazinam in rats:

Reference.: *Beck M.; 2006;* Report No. WIL-282006

The study was conducted according to US EPA OPPTS Guideline 870.3700 and OECD Guideline 414 and is in compliance with GLP. The study is considered acceptable.

Material and method:

3 groups of 25 mated female rats (strain: Crl:CD (SD); source: Charles River, Raleigh, North Carolina), approximately 70 days old at commencement of the study, received oral doses (gavage) containing 10, 50 and 300 mg/kg bw fluazinam (batch Lot A629/1995, purity 97.3 %) from day 6 to 19 of gestation. 25 animals served as controls, receiving the vehicle (0.5 % carboxymethylcellulose sodium) by gavage. Diets were prepared daily; concentrations of fluazinam in the diet, stability and homogenicity of the test substance were confirmed by analysis. Animals were checked twice daily for mortalities or signs of reaction. Individual body weights were recorded on days 0 and 6 – 20 (daily), group mean body weights were calculated for each of these days. Food consumption was recorded for each animal on days 0 and 6 – 20 (daily).

On day 20 post coitum, a laparohysterectomy was performed on each female. The contents of the thoracic, abdominal and pelvic cavities were examined and the livers weighed. The reproductive tract was examined and the numbers of foetuses, early and late resorptions, total implantations, corpora lutea and placental weights were recorded. Gravid uterine weights were recorded and net body weight (excl. uterus + contents) and net body weight change were calculated. Foetuses were removed, sexed, weighed and examined for external, visceral and skeletal malformations and developmental variations.

Findings:

The general condition of the treated females was similar to that of the controls throughout the study. Animals of the 300 mg/kg bw/d dose group showed statistically significant weight loss between days 6 – 9. Mean food consumption of animals treated with 300 and 50 mg/kg bw/d fluazinam was reduced during the early dosing period (gestation days 6 – 9), followed by a reduced rate of weight

gain compared to controls. Animals of the high dose group showed reduced mean body weight gain during gestation days 15 – 20 also, attributed to the decreased mean gravid uterine weight that corresponded to a decrease in the mean number of viable foetuses and reduced mean fetal weights. Mean food consumption of animals treated with 300 mg/kg bw/d continued to be lower than controls for the remainder of the treatment period, mean body weights were lower from gestation days 8 – 20.

At 10 mg/kg bw fluazinam, food consumption, body weights and body weight gains were similar in comparison to the concurrent control values throughout the study.

Mean maternal body weights (g) during gestation

Dose	Days post coitum				
Mg/kg	0	6	9	15	20
0	252	284	293	322	394
10	252	283	290	319	385
50	252	283	288	315	382
300	254	285	280*	307**	359**

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

Mean maternal body weights, gravid uterine weights, net body weights and net body weight changes (g)

	Initial bw	Terminal bw	Gravid uterin weight	Net bw	Net bw change
0	252	394	85	308.8	56.4
10	252	385	78.4	307.0	55.5
50	252	382	79.3	302.6	50.1
300	254	359**	65.9**	292.9*	39.4**

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

Reproduction data:
Test article-related effects on intrauterine growth and/or survival were noted in the 50 and 300 mg/kg bw groups. Mean number and litter proportion of viable foetuses in the 300 mg/kg bw group were statistically significantly lower than controls due to an increase in the mean litter proportion of postimplantation loss (early resorptions). Mean fetal body weights were statistically significantly reduced in the 50 and 300 mg/kg bw groups. Mean placental weights, numbers of corpora lutea, implantation sites and mean litter proportion of preimplantation loss were similar to controls in all dose groups.

Group mean litter data for female rats treated with fluazinam (% per litter)

	Dose (mg/kg/bw/d)	0	10	50	300
	Corpora lutea count	17.3	17.1	17.1	17.2
	Implantation sites	15.9	15.6	16.4	15.6
	Viable foetuses	96.4	94.4	93.3	85.8*
	Resorptions total	3.6	5.6	6.7	13.9
	early	3.6	5.6	6.0	11.5
	late	0.0	0.0	0.7	2.4
	Preimplantation loss (%)	7.6	9.0	3.5	8.1
	Postimplantation loss (%)	3.6	5.6	6.7	14.2*
	Fetal weight (g)	3.6	3.5	3.4*	3.0**
	Placental weight (g)	0.44	0.43	0.43	0.43

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

External malformations were noted in 1(1), 0(0), 3(3) and 4(3) foetuses (litters) in the control, 10, 50 and 300 mg/kg bw/d groups, respectively.
Two foetuses of the 300 mg/kg bw/d group had a bent tail (0.6 %), one of them a bilateral microphthalmia (0.3 %). Two foetuses of this dose group showed edema of the thorax. In the 50 mg/kg bw/d group, tarsal flexure, fetal anasarca and omphalocele were noted in 3 foetuses, respectively. Due to the low mean litter proportions of these findings, the lack of statistical significance and the occurrence of the findings within historical control data range, all external malformations in the 50 and 300 mg/kg bw/d groups were considered unrelated to treatment.

Visceral malformations and variations:
Mean litter proportions of renal papillae not developed and/or distended ureter(s) in the 50 and 300 mg/kg bw/d groups (1.6 % and 2.5 % per litter, respectively) were increased compared to concurrent controls (0.8 % per litter). Although the differences were not statistically significant compared to the concurrent controls, the values exceed the maximum mean value in the historical control data (0.8 % per litter). A dose-related increase of renal papillae not fully developed were observed in 2(1) and 5(4) foetuses (litters) in the 50 and 300 mg/kg bw/d groups, respectively.

Skeletal malformations and variations:
Test article related differences in mean litter proportions of skeletal developmental variations (unossified sternebrae, reduced ossification of the skull, cervical centrum and vertebral arches) were noted in the 50 and 300 mg/kg bw/d groups, though not statistically significant compared to concurrent controls. However, these developmental variations were considered test article related because they corresponded to the reduced mean fetal body weights in the 50 and 300 mg/kg bw/d groups, indicating a developmental delay and/or were outside the historical control data range. Mean litter proportion of 27 presacral vertebrae in the 300 mg/kg bw/d group (3.2 % per litter) was higher than concurrent controls (0.0 % per litter) and outside the historical control data range (1.8 % per litter), though not statistically significant.

Fetal observations at external, visceral and skeletal examination (number of litters)

	Dose (mg/kg/day)				
Parameter	0	10	50	300	Historical control data ranges (76 studies)
Foetuses (litters)	384 (25)	367 (25)	367 (25)	319 (25)	27453 (1805)
Renal papillae not developed and/or distended ureters	3 (2) [0.8%]	0 (0) [0.0%]	6 (5) [1.6%]	7 (4) [2.5%]	6 (5) [0 - 0.8%]
Sternebra(e) 5 and/or 6 unossified	82 (18) [20.8%]	66 (20) [17.3%]	140 (23) [37.4%]	120 (22) [39.7%]	2237 (750) [0.3 – 23.1%]
Sternebra(e) 1, 2, 3 and/or 4 unossified	2 (2) [0.5%]	1 (1) [0.3%]	0 (0) [0.0%]	5 (3) [1.7%]	59 (53) [0.0 – 1.3%]
Reduced ossification of skull	0 (0) [0.0%]	2 (1) [0.6%]	15 (4) [4.1%]	42 (8) [14.4%]	1 (1) [0.0 – 1.0 %]
Cervical centrum ossified	91 (21) [22.8%]	91 (19) [24.6%]	82 (19) [22.1%]	35 (14) [10.8%]	5104 (1344) [6.6 – 32.1%]
Reduced ossification of vertebral arches	0 (0) [0.0 %]	1 (1) [0.3 %]	0 (0) [0.0 %]	4 (2) [1.2 %]	11 (11) [0.0 – 0.8%]
27 presacral vertebrae	0 (0) [0.0 %]	0 (0) [0.0 %]	2 (2) [0.5 %]	7 (6) [3.2 %]	37 (27) [0.0 – 1.8%]

Conclusion:
Indications of maternal toxicity consisted of lower mean food consumption and lower mean body weight gains in the 50 and 300 mg/kg bw/d groups, animals of the 300 mg/kg bw/d group showed weight loss between gestation days 6 – 9 also. A statistically significant reduced mean body weight gain during gestation days 15 – 20 was observed in animals of the high dose group, primarily due to the decreased mean gravid uterine weight that correlated with reduced mean fetal weights and a decrease in the mean number of viable foetuses.

The NOAEL for maternal toxicity was considered to be 10 mg/kg bw/d.

Developmental toxicity was expressed in the 50 and 300 mg/kg bw/d groups. Mean litter proportion of postimplantation loss (early resorptions) in the 300 mg/kg bw group was statistically significantly higher than controls. This resulted in a statistically significant decrease in the mean number and mean litter proportion of viable foetuses.

Mean fetal body weights were statistically significantly reduced in the 50 and 300 mg/kg bw groups. In this dose groups, an increase of renal papillae not developed and/or distended ureter(s) were observed (1.6 % and 2.5 % per litter), although not statistically significant compared to concurrent controls, but the values exceeded the maximum mean value in the historical control data

(0.8 %). Test article-related skeletal variations included increases of reduced ossification of the skull and vertebral arches, unossified sternebrae and a decrease of ossified cervical centrum no. 1. There was no indication of teratogenicity in this study.
The NOAEL for developmental effects can be set at 10 mg/kg bw/d.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

No data available.

4.11.4 Summary and discussion of reproductive toxicity

In a two generation reproduction study, rats fed a diet containing fluazinam in the highest concentration of 500 ppm showed statistically significant reductions in body weight and body weight gain and reduced food intake. Relative liver weights were significantly increased in both sexes of the highest dose group and also in females of the intermediate group of the F_0 generation. Relative liver weights in F_1 adults were increased in males of the highest dose group and in males of the intermediate group also. Histopathologically, an statistically significant increase of periacinar hepatocytic fatty change was detected in high dose males of F_0 and F_1 animals and also in F_1 males of the 100 ppm group. The **NOAEL for systemic toxicity** was considered to be **20 ppm**, equivalent to approximately **1.5 mg/kg bw/d for males** and **1.7 mg/kg bw/d for females**. Reproductive performance of F_0 animals was unaffected by treatment. In the F_1 generation, conception rate and fertility index were slightly reduced in the 500 ppm group. Gestation length was slightly increased in the high and intermediate dose groups, but not statistically significant. Numbers of implantation sites and mean litter sizes to day 4 post partum were statistically significantly reduced for F_1 animals of the high dose group and marginally lower in the intermediate group (100 ppm). As implantation sites and litter sizes at 100 ppm in the second generation were not statistically significantly reduced, the **reproductive NOAEL** can be changed to **100 ppm**, equivalent to approximately **7.26 mg/kg bw/d for males and 8.43 mg/kg bw/d for females**.

Two teratology studies in rabbits had been performed. In the first study, dose levels of 0.3, 1 and 3 mg/kg bw fluazinam from day 6 to 19 of gestation had been chosen. There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d). As the mean food consumption of animals treated with 3 mg/kg bw/d fluazinam was slightly, but not statistically significantly reduced, the **maternal NOAEL** can be changed to **3 mg/kg bw/d**. Based on incomplete ossification in the high dose group (twice as much of the control group), the **NOAEL for fetal toxicity** is **1 mg/kg bw/d**.

In the second study, oral administration of fluazinam to pregnant rabbits during the period of organogenesis was associated with reduced maternal weight gain and food intake in the highest dose group of 12 mg/kg bw/d. Macroscopic and microscopic lung and liver changes reached statistical significance at a dose level of 7 mg/kg bw/d. So the **maternal NOAEL** can be changed to **4 mg/kg/day**.
Increased incidences of fetal abnormalities (placental abnormalities, some skeletal abnormalities including kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were seen at the top dose. At all dose levels, increased incidences of preimplantation losses were observed, however, the values fell within the recorded background control range of the

laboratory. Postimplantation loss was increased at 4 mg/kg/day compared to concurrent controls, however, no increase was observed at the 7mg/kg/day dose level. As statistical significance was only reached at a dose level of 12 mg/kg bw/d, the **NOAEL for fetal toxicity** can be changed to **7 mg/kg bw/d**.

In the first teratology study in rats, oral administration of fluazinam at the high dose level of 250 mg/kg bw/d to pregnant rats during the period of organogenesis was associated with reduced mean food consumption and weight loss, followed by a slight reduced rate of weight gain compared to controls. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. So the **maternal NOAEL** was considered at **10 mg/kg/day**.
Fetal and placental weights were significantly reduced in the high dose group and there were indications of fetal immaturity. In the 50 mg/kg bw/d group, fetal and placental weights were reduced, but not significantly, compared to controls. An increased incidence of gross morphological fetal abnormalities were recorded at the top dose, values were outside the range of the concurrent controls and the recorded background controls of the laboratory. In this study, fluazinam showed a teratogenic potential at a maternal toxic dose of 250 mg/kg bw/d after oral application. The **NOAEL** for developmental effects was considered at **10 mg/kg bw/d**.
In a second teratology study in rats, maternal toxicity consisted of lower mean food consumption and lower mean body weight gains in the 50 and 300 mg/kg bw/d groups. Animals of the 300 mg/kg bw/d group showed weight loss also. Due to decreased mean gravid uterine weights, reduced mean fetal weights and decreased mean numbers of viable foetuses, a statistically significant reduced mean body weight gain during gestation days 15 – 20 was observed in animals of the high dose group.

The **NOAEL** for maternal toxicity was considered to be **10 mg/kg bw/d**.

Developmental toxicity was observed in the 50 and 300 mg/kg bw/d groups. In the high dose group, postimplantation loss was statistically significantly higher than controls, resulting in a statistically significant decrease in mean number and mean litter proportion of viable foetuses.

Mean fetal body weights were statistically significantly reduced in the 50 and 300 mg/kg bw groups. In this dose groups, not developed renal papillae and/or distended ureter(s) were observed, although not statistically significant compared to concurrent controls, but the values exceeded the maximum mean value in the historical control data. Test article-related skeletal variations included increases of reduced ossification of the skull and vertebral arches, unossified sternebrae and a decrease of ossified cervical centrum no. 1.
The **NOAEL for developmental effects** can be set at **10 mg/kg bw/d**.
According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam should be classified to "category 3 of reproductive substances" and labelled with the risk phrase "R 63 – Possible risk of harm to the unborn child".

4.11.5 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as Xn, Toxic to reproduction category 3, R63 (Possible risk of harm to the unborn child) and in hazard category 2 for reproductive toxicity and labeled with signal word "Warning" and hazard statement H361 (Suspected of damaging the unborn child), respectively for the following reasons:
In a teratology study in rabbits, increased incidences of fetal abnormalities (placental abnormalities, kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were observed.
In a teratology study in rats, fetal and placental weights were significantly reduced, fetal immaturity

and gross morphological fetal abnormalities were reported. In a second study in rats, postimplantation loss, resulting in a statistically significant decrease of viable foetuses was reported. Decreased fetal weight, not developed renal papillae, distended ureter(s), reduced ossification of the skull and vertebral arches and unossified sternebrae were observed.

4.11.6 Conclusions on classification and labelling

According to Annex VI of the EC Council Directive 67/548/EEC, fluazinam has to be classified as Xn, Toxic to reproduction category 3, R63 (Possible risk of harm to the unborn child).

According to Regulation EC 1272/2008, fluazinam should be classified in hazard category 2 for reproductive toxicity and labeled with signal word “Warning” and hazard statement H361 (Suspected of damaging the unborn child).

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

Five relevant reproductive toxicity studies are presented:

- A two generation reproduction study (Tesh *et al*, 1987) for effects on fertility
- Two teratology studies in rabbits (Tesh *et al*, 1985, 1988) for developmental toxicity
- Two teratology studies in rats : (Willoughby *et al*, 1984) and (Beck, 2006) for developmental toxicity

1. Sexual function and fertility

Two generation reproduction study in rats (Tesh et al, 1987)

F1 and F2 male and female rats received diets containing 0, 20, 100 or 500 ppm of fluazinam.

Body weights were recorded weekly through mating and on gestation days 0, 6, 13 and 20 and lactation days 1, 4, 7, 14 and 21 in females. The oestrus cycle, mating performance and fertility were recorded. Offspring was observed for clinical signs and mortality and body weights were recorded on days 1, 4, 7, 11, 14 and 21 after birth. Physical development was assessed on a litter basis based on pinna unfolding, hair growth, tooth eruption and eye opening.

Findings:

Body weights

For both generations and both sexes, mean food consumption of treated animals of the low (20 ppm) and intermediate groups (100ppm) was not different compared to controls.

F_0 females and both sexes of the F_1 generation of the high dose group (500 ppm) showed a slight reduction in food intake during maturation. Mean body weight of F_0 females of the 500 ppm group was reduced during maturation (9.4 %) and early gestation periods (6 to 7 %). Throughout the lactation period, mean body weight was similar to that of controls.

Mean body weight was significantly reduced for females of the F_1 generation receiving 500 ppm of fluazinam during the maturation (4.5 to 12.2 %) and gestation periods (12 to 13 %). Mean body weight of females of the intermediate group (100 ppm) was slightly reduced (2 to 4 %) during the gestation period when compared to controls. Reduced mean body weight was also recorded in F_1 females of the 500 ppm group during the gestation and lactation period (10 to 11 %).

Mating performance

Mating performance, pregnancy rate and gestation index of the F_0 generation were not adversely affected by treatment at any dose level. Gestation length was very slightly increased (23 days versus 22.5) in the high dose group. Implantation sites and mean litter sizes were within the laboratory background control ranges.

In the F_1 generation, conception rate and fertility index were slightly reduced (75 % versus 91 % and 75 % vs. 87 %, respectively) in the 500 ppm group compared to the control group. Gestation length was slightly increased (23 days versus 22.5) in the high (500 ppm) and intermediate (100 ppm) dose groups. Numbers of implantation sites (12.2 versus 15.3) and mean litter sizes up to day 4 post partum (9.8 versus 12.4) were slightly reduced for F_1 animals of the high dose group (500 ppm) and the intermediate group (100 ppm; 13.1 and 11.3 versus 15.3 and 12.4, respectively), but not statistically significant. In both generations, survival and lactation indices and sex ratios were unaffected by treatment.

Offspring

Birth weight of F_1 pups was similar in all groups but the mean body weight gain of pups during lactation period was significantly lower in the 500 ppm group compared to the control group at weaning (44.5 g versus 50.8 g) despite the culling that occurred on day 4 post partum. The rate of physical development (pinna unfolding, hair growth, tooth eruption and eye opening) of F_1 offspring was similar in all dose groups, although completion of these developmental landmarks was slightly earlier in the 500 ppm group (statistically significant for pinna unfolding, hair growth and eye opening).

Pathology

Necropsy of adults and offspring in both generations revealed no adverse treatment related effects.

Increased absolute liver weights, although not statistically significant, were seen in F_0 females of all treated groups and in F_0 males (23.3 g versus 22.3g) and females (14.0 g versus 13.5 g) receiving 500 ppm. Relative liver weights were significantly increased in both sexes in the highest dose groups (7.6 % for males and 12.1 % for females when compared to controls), as well as in females in the intermediate (5 %) and low dose (5.2 %) group, but a clear dose response was not observed.

Histopathology

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

The **NOAEL for systemic toxicity** was considered to be **20 ppm**, equivalent to approximately **1.5 mg/kg bw/d for males** and **1.7 mg/kg bw/d for females**.

Therefore, the reproductive NOAEL can be set at 100 ppm, equivalent to approximately 7.26 mg/kg bw/d for males and 8.43 mg/kg bw/d for females.

2. Developmental toxicity

Two teratology studies in rabbits (Tesh et al, 1985, 1988)

In the **first study** (1985) results should be cautiously considered since several animals were affected by a Pasteurella infection. In this study, rabbits were exposed at dose levels of 0.3, 1 and 3 mg/kg bw fluazinam from day 6 to 19 of gestation.

There was no evidence of a teratogenic potential up to the highest dose tested (3 mg/kg bw/d). As the mean food consumption of animals treated with 3 mg/kg bw/d fluazinam was slightly, but not statistically significantly reduced, the **maternal NOAEL** can be set at **3 mg/kg bw/d**.

Based on incomplete ossification in the high dose group (incidence twice as high compared to the control group), the **NOAEL for foetal toxicity** is **1 mg/kg bw/d**.

In the **second study** (1988), the general condition of the treated white rabbits females exposed at 2, 4, 7 and 12 mg/kg was similar to that of the controls throughout the study.

Findings:

Body weights

Absolute maternal body weights in animals dosed at concentrations of 2, 4 and 7 mg/kg/day were comparable to controls. Mean body weights in 12 mg/kg/day dosed animals were lower than concurrent controls from day 10 through day 20 of gestation, reaching statistical significance on day 20 (4.07 kg versus 4.33 kg). The mean body weights were increased during the post-dosing period and the animals had recovered approximately 50 % of their body weight gain differences with the concurrent control animals by termination (4.25 kg versus 4.40 kg)

Necropsy findings

- *Macroscopic examination* showed respiratory tract infection and areas of discolouration or pallor of livers in animals of the 4, 7 and 12 mg/kg bw/d groups.
- *Microscopic changes* included: hepatocytic hypertrophy at 7 and 12 mg/kg; increased apoptosis (2 animals out of 16); necrosis/degeneration of single hepatocytes (2 and 4 animals out of 16); increased brown pigment within the hepatocytes (3 and 2 animals out of 16); focal hepatocytic necrosis (0 and 2 animals /16); bile plugs (1 animal / 16 at the highest dose); and an increase in the number of binucleate hepatocytes. Statistical significance was reached in the 7 and 12 mg/kg bw/d groups.

So the **maternal NOAEL** can be set at 4 mg/kg/day.

Reproduction data

Abortion

Two females (out of 17) in each of the 4 and 7 mg/kg bw/d dose groups and one out of 17 animals in the 12 mg/kg bw/d group aborted during the study. Total resorption was observed in one animal out of 17 in the 7 mg/kg bw/d group and in 5 animals out of 16 in the 12 mg/kg bw/d group.

Pre-implantation loss was elevated without dose-response relationship (between 19.8 % and 27.2 %) in all treated groups in comparison to the concurrent controls, but all values fell within the recorded background control range of the laboratory (4.7 – 35.7 % in 92 studies).

Post-implantation loss was increased at 4 mg/kg/day (25.9 %) compared to concurrent controls, however, no increase was observed at the 7mg/kg/day dose level. A significant post-implantation loss (20 %) was noted for the 12 mg/kg bw/d group.

There were placental anomalies (not described) that exceeded the historical control high values in the 12 mg/kg/day group (18.2 %).

Foetal observations

There were several abnormalities noted in foetuses during the external and visceral examination of all treatment groups, but mainly in the high-dose group.

The incidence of several skeletal abnormalities was clearly increased in the high-dose group compared to both the study control values and the historical control range for the laboratory. Effects that may be treatment related include kinked tail tip (4.5 %), fused (9.1 %) or incompletely (2.3 %) ossified sternebrae and abnormalities of the head bones (6.8 %)

As significance can be reached at a dose level of 12 mg/kg bw/d, **the NOEL for foetal toxicity can be set at 7 mg/kg bw/d.**

Teratology study in rats (Willoughby et al, 1984)

Female rats received oral doses (gavage) containing 10, 50 and 250 mg/kg bw fluazinam from day 6 to 15 of gestation.

Findings:

Body weights

Animals dosed with 250 mg/kg/day showed weight loss between 6 and 8 days post coitum and statistically significant reduced weight gain when compared to controls during the treatment period (-41.2 % less body weight gain than the control group) which persisted until the end of the gestation (-15.5 % less body weight gain than the control group). This effect on the body weight at the top dose was associated with a statistically significant reduction in the mean food consumption during the early dosing period Weight gain in the 50 mg/kg bw/d group was marginally, but not significantly, reduced. So the **maternal NOAEL** was considered to be **10 mg/kg/day.**

Necropsy findings

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

Reproduction data

– Numbers of implantations, live young and the extent of pre-implantation loss were unaffected by treatment with fluazinam.

– Post-implantation loss was increased (11 % versus 4.2 %) in the 250 mg/kg/day group compared to concurrent controls, however, not statistically significant and within the range of the historical controls of the laboratory.

– Foetal and placental weights were significantly reduced in the high dose group (250 mg/kg)n (2.81 g versus 3.19 g and 0.47 g versus 054 g respectively) These reductions were also seen at the intermediate dose level (50 mg /kg bw/d) but without statistical significance (3.11 g and 0.49 g respectively). The 10 mg/kg/day dose group was unaffected by treatment with fluazinam.

– Abnormalities were noted in the litters of four high-dose animals and included facial/palatal cleft and/or diaphragmatic hernia. Three litters had just one foetus with one of the abnormalities and the remaining litter with up to 8 foetuses with facial/palatal cleft.

– The skeletal examination showed a reduction in the degree of ossification of cranial bones (54.3 % versus 22.9 %), sternebrae, caudal vertebrae (13 % versus 6.4 %), metacarpals/metatarsals (10.1 % versus 7.1 %) and pubic bones in high-dose foetuses (22.5 % versus 10.7 %). They were outside of the historical control.

– An increased incidence of gross morphological fetal abnormalities (diaphragmatic hernia (3.1 %) and facial/palatal cleft (2.3 %) was recorded at the top dose, values were outside the range of the concurrent controls and the recorded background controls of the laboratory.

In this study, fluazinam showed a teratogenic potential at a maternally toxic dose of 250 mg/kg bw/d after oral application. The **NOAEL** for developmental effects was considered to be **10 mg/kg bw/d**.

Teratology study in rats (Beck, 2006)

Mated females rats received oral doses (gavage) containing 10, 50 and 300 mg/kg bw fluazinam from day 6 to 19 of gestation.

Findings:

Body weights

Pregnant females of the 300 mg/kg bw/d group lost weight between day 6 and 9 of pregnancy (-1.8 %), and the mean body weight of this group remained statistically significantly inferior compared to the control group from day 9 to day 20 of pregnancy (4.4 % on day 9 of gestation to 8.9 % on day 20 of gestation). Due to decreased mean gravid uterine weights, reduced mean foetal weights and decreased mean numbers of viable foetuses, a statistically significant reduced mean body weight gain during gestation days 15 – 20 was observed in animals of the high dose group. The mean net body weight gain of the high dose group was therefore 30.1 % below the value of the control group. At 50 mg/ kg/day, the terminal net mean body weight of females was also reduced when compared to control (11.2 %) but without statistical significance.

The **NOAEL** for maternal toxicity was considered to be **10 mg/kg bw/d**.

Reproduction data

Mean litter data for the different treatment groups showed that the percentage of viable foetuses in the litters in the 300 mg/kg bw/d group were statistically significantly lower (85.8 versus 96.4 %) than controls due to an increase in the mean litter proportion of post-implantation loss (early resorptions) (14.2 % versus 3.6 %; statistically significant). Mean foetal body weights (3.4 and 3.0 g versus 3.6 g) were statistically significantly reduced in the 50 and 300 mg/kg bw/day groups. Mean placental weights, number of corpora lutea, implantation sites and mean litter proportion of pre-implantation loss were similar to controls in all dose groups.

External malformations

External malformations were noted in the control, 10, 50 and 300 mg/kg bw/d groups, respectively. Due to the low mean litter proportions of these findings, the lack of statistical significance and the fact that the occurrence of the findings were within historical control data range, all external malformations in the 50 and 300 mg/kg bw/d groups were considered unrelated to treatment.

Visceral malformations and variations

Mean litter proportions of renal papillae not developed and/or distended ureter(s) in the 50 and 300 mg/kg bw/d groups (1.6 % and 2.5 % per litter, respectively) were increased compared to concurrent controls (0.8 % per litter). Although the differences were not statistically significant compared to the concurrent controls, the values exceed the maximum mean value in the historical control data (0.8 % per litter). A dose-related increase of renal papillae not fully developed were observed in 2(1) and 5(4) foetuses(litters) in the 50 and 300 mg/kg bw/d groups, respectively.

Skeletal malformations and variations

Treatment related differences in mean litter proportions of skeletal developmental variations (unossified sternebrae, reduced ossification of the skull, cervical centrum and vertebral arches) were noted in the 50 and 300 mg/kg bw/d groups, although not statistically significant compared to concurrent controls. However, these developmental variations were considered treatment related because they corresponded to the reduced mean fetal body weights in the 50 and 300 mg/kg bw/d groups, indicating a developmental delay and/or were outside the historical control data range.

Mean litter proportion of 27 pre sacral vertebrae in the 300 mg/kg bw/d group (3.2 % per litter) was higher than concurrent controls (0.0 % per litter) and outside the historical control data range (1.8 % per litter), although not statistically significant.

The **NOAEL for developmental effects** can be set at **10 mg/kg bw/d**.

Dossier submitter's classification proposal:

Considering the criteria for classification and labelling according to Dir 67/548/EEC and the CLP Regulation, fluazinam should be classified as Repr. Cat. 3; R63 (Possible risk of harm to the unborn child) and Repr. 2 (H361) and labelled with the signal word "Warning", respectively, for the following reasons:

In a teratology study in rabbits, increased incidences of fetal abnormalities (placental abnormalities, kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were observed.

In a teratology study in rats, fetal and placental weights were significantly reduced, fetal immaturity and gross morphological fetal abnormalities were reported. In a second study in rats, post-implantation loss, resulting in a statistically significant decrease of viable foetuses was reported. Decreased fetal weight, not developed renal papillae, distended ureter(s), reduced ossification of the skull and vertebral arches and unossified sternebrae were observed.

Comments received during public consultation

– Five MSCAs supported the classification proposed by the dossier submitter.

– One MSCA considered that the occurrence of palatal clefts and diaphragmatic hernia in rat foetuses at a dose level of 250 mg/kg bw (Willoughby *et al*. 1984), significant signs of foetal growth retardation in rats at a dose level of 300 mg/kg bw (Beck *et al.* 2006), and high resorption rates in rats at a dose level of 300 mg/kg bw (Beck *et al.* 2006), would justify a classification for reproductive toxicity in **Category 2; R61** (Directive 67/548/EEC) and **Category 1B (H360D)** (CLP Regulation), respectively. On the other hand, cleft palates and diaphragmatic hernia were not observed in the study by Beck *et al*. (2006) up to a dose level of 300 mg/kg bw/d and the observed findings in foetuses need to be balanced against maternally toxicity.

- The sole notifier in Europe commented on the classification and disagreed with the dossier submitter's proposal. The notifier provided the study of Beck, 2006, which was assessed by the dossier submitter. The notifier's rationale for no classification is based on the two teratogenicity studies in rat. The first study conducted in 1985 used corn oil as vehicle. The quality was not described and it is suggested that the difference of results compared to the second study could be linked to the vehicle.

RAC assessment - comparison with the classification criteria and justification

Comparison with the criteria:

Considering the criteria for classification and labelling, fluazinam should be classified as Repr. 2 (H361d) according to the CLP Regulation (Repr. Cat. 3; R63, according to Directive 67/548/EEC) for the following reasons:

In a teratology study in rabbits, increased incidences of foetal abnormalities (placental abnormalities, kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones) were observed at the top dose (12 mg/kg). The effects were seen in presence of maternal toxicity and were outside the range of historical of control values.
In a teratology study in rats, foetal and placental weights were significantly reduced, foetal immaturity and gross morphological foetal abnormalities were reported at maternally toxic doses. In this study, impact on the foetal development of the vehicle used (corn oil) cannot be dismissed.

In a second study in rats, post-implantation loss, resulting in a statistically significant decrease of viable foetuses, was reported. Decreased foetal weight, not developed renal papillae, distended ureter(s), reduced ossification of the skull and vertebral arches and unossified sternebrae were observed at 300 mg/kg in presence of maternal toxicity.

There is no reason to increase the classification to Repr. 1B according to the CLP Regulation (Repr. Cat 2; R61 according to Dir 67/548/EEC), since the adverse effects on development were seen in both species only at dose levels where also maternal toxicity was seen and since the effect of the vehicle on the occurrence of severe abnormalities cannot be dismissed in one rat study.

To complete the analysis of the studies, the two generation study on rats shows that there is no effect on fertility and no effects on the postnatal development of the pups related to the fluazinam toxicity.

Reproductive toxicity category 2 in the CLP Regulation is dedicated to substances which are "suspected human reproductive toxicants". "Substances are classified in category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where there the evidence is not sufficiently convincing to place the substance in category 1."

The incidence of abnormalities, their type and distribution among litters and species do not provide strong enough evidence of "known" teratogenicity to fulfil the CLP category 1B criteria as reminded below:

Reproductive toxicity category 1 in the CLP Regulation is dedicated to "substances which are known or presumed human reproductive toxicant". Substances are classified in category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility or on development in humans or when there is evidence from animal studies possibly supplemented with other information, to provide as strong presumption that the substance has the capacity to interfere with reproduction with humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (category 1A) or from animal data (category 1B).

Conclusion:

When comparing the available data with the classification criteria, RAC concluded that Repr. 2 (H361d) according to the CLP Regulation (Repr. Cat. 3; R63 according to Directive 67/548/EEC) is justified.

4.12 Other effects

No data available.

4.12.1 Non-human information

No data available.

4.12.1.1 Neurotoxicity

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

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

4.12.1.2 Immunotoxicity

No data available.

4.12.1.3 Specific investigations: other studies

No data available.

4.12.1.4 Human information

No data available.

4.12.2 Summary and discussion

No evidence of neurotoxicity and neuropathology was observed after single oral doses and after 13 weeks of treatment with fluazinam.

4.12.3 Comparison with criteria

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, no classification for Fluazinam considering neurotoxic and neuropathologic effects is considered necessary.

4.12.4 Conclusions on classification and labelling

No classification is required considering neurotoxic and neuropathologic properties.

RAC evaluation of neurotoxicity / neuropathology

Summary of the Dossier Submitter's proposal

No evidence of neurotoxicity and neuropathology was observed after single oral doses and after 13 weeks of treatment with fluazinam.

Considering the criteria for classification and labelling according to Directive 67/548/EEC and the CLP Regulation, no classification for fluazinam for neurotoxic and neuropathologic effects is considered necessary.

Comments received during public consultation

No comments were received during public consultation.

RAC assessment - comparison with the classification criteria and justification

Conclusion:

As no evidence of neurotoxicity and neuropathology was observed fluazinam does not meet the CLP criteria for classification.

5 ENVIRONMENTAL HAZARD ASSESSMENT

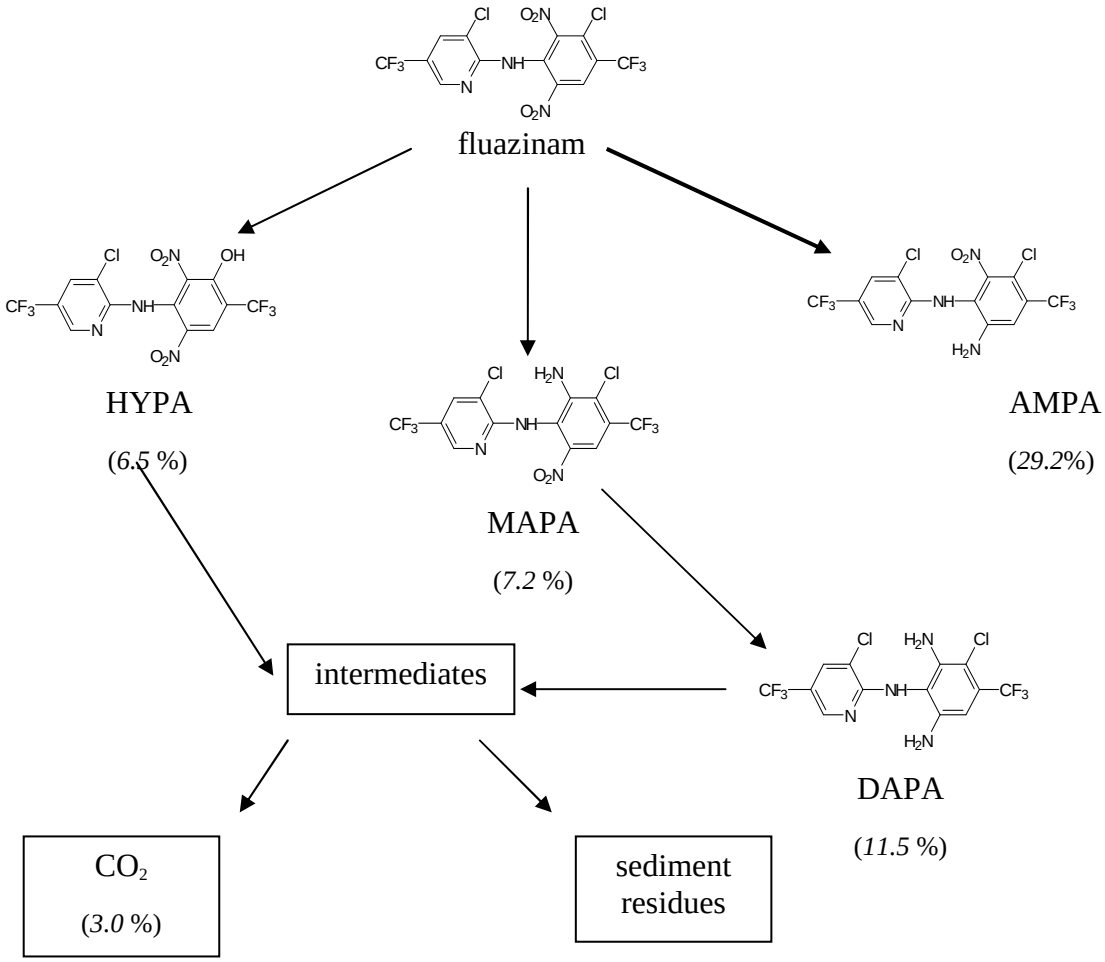
5.1 Degradation

Table 19: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis 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 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	van der Gaauw, A. (2003) (Document 846211)
Photolysis 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	Lentz, N.R., Korsch, B.H. (1995) (Document 5312-94-0119-EF-002)

C

Method	Results			Remarks	Reference
Ready biodegradability Manometric Respirometry Test OECD 301F; EU EEC, C.4-D	fluazinam is not readily biodegradable.			Acceptable	Grützner 2000 (Report No. 774898)
Water/Sediment study Guideline: BBA Guidelines, Part IV, Section 5-1; proposed UK Guidelines for the Conduct of Biodegradability Tests on Pesticides in Natural Sediment-Water Systems (1992)	DT_{50} / DT_{90} whole sys. (days) 3.1 / 33.7 ***** RMS: Phenyl: 4.3 / 14.2 Pyridyl: 4.6 / 15.2 ***** Timme/Frehse (square root 1st order)!	DT_{50} / DT_{90} water (days) RMS: Phenyl: 1.9 / 6.3 Pyridyl: 3.5 / 11.8	DT_{50} / DT_{90} sediment (days) RMS: Phenyl: 4.4 / 14.7 Pyridyl: 6.4 / 21.3	Acceptable	Goodyear 1997 (Report No. 38/188-1015)



Proposed metabolic pathway of fluazinam in water/sediment systems
(*values in bracketc: maxima observed in the whole system for a single label position*)

5.1.1 Stability

Hydrolysis of active substance

Reference: van der Gaauw, A. (2003): ^{14}C-Fluazinam: Hydrolysis at Three Different pH Values. (RCC study no. 846211)

Guideline: OECD 111; 92/69/EEC part C.7; EPA OPPTS 835.2110; SETAC (Europe) Part 9

GLP: yes

Test item: [^{14}C-Phenyl] Fluazinam, radiochemical purity: 100%, batch no.: 96-J29; [2,6-^{14}C-Pyridine] Fluazinam, radiochemical purity: 97.7%, batch no.: 96J30

Material and methods:

The abiotic hydrolysis of ^{14}C-labelled fluazinam (concentration: 0.04 – 0.05 mg/L) was investigated in sterile aqueous buffer solutions at pH 4, 7 and 9. Incubation at pH 4 was performed at 50 °C for up to 5 days, whereas for pH 7 and 9 it was conducted at 25°C and 50°C for up to 29 or 56 days. During the incubation time periodically, the pH of each buffer solution was recorded and test samples were taken and analysed by LSC (total radioactivity), HPLC and TLC (radioactive fractions).

Findings:
All test solutions remained sterile and no significant variation of temperature and pH value was observed throughout the study. Mean recoveries of total radioactivity for both labels were between 95.8 ± 5.0 % (pH 4, 50°C) and 103.6 ± 2.4 % (pH 7, 50°C).
At pH 4 fluazinam was not degraded by hydrolysis. After 5 days at 50 °C, mainly unchanged parent was found for both labels in respective test samples.
At pH 7, fluazinam was rapidly hydrolyzed. CAPA was the only hydrolysis product formed at 25 °C, representing 92.3% (label I) and 95.1% (label II) of the applied radioactivity after 29 days. At 50 °C the major metabolite CAPA was steadily hydrolyzed to DCPA with a DT_{50} value of about 32 days. At the end of incubation DCPA was found in amounts of 70.9 % (label I, day 56) and 38 % (label II day 28) of the applied radioactivity. DCPA was resistant to further degradation. For both labels, an additional minor hydrolysis product was detected at a maximum level of 5 % on day 29.
At pH 9, hydrolysis of fluazinam was similarly rapid comparable to that at pH 7. CAPA was again the major hydrolysis product formed at 25 °C, representing 94.0% (label I) and 102.6% (label II) of the applied radioactivity at the end of incubation (day 29). At 50 °C CAPA was steadily hydrolyzed to DCPA with a DT_{50} value of about 8 days. DCPA represented 95.5% and 95.4% of the applied radioactivity for labels I and II, respectively, at day 29. No further degradation of this major metabolite was observed.

Table 20: Balance and distribution of radioactivity in the buffer solutions (in % AR) at 25 °C (phenyl label/pyridyl label)

days	fluazinam	CAPA	total	days	fluazinam	CAPA	DCPA	total
	pH 7				pH 9			
0	94.0 / 100.0	Nd / nd	94.0 / 100.0	**0**	97.4 / 100.0	2.6 / nd	Nd	100.0 / 100.0
2	55.5 / -	38.9 / -	94.4 / -	**1**	77.2 / 88.6	23.9 / 12.2	Nd	101.1 / 100.9
5	40.9 / 27.5	57.6 / 72.3	98.5 / 99.9	**2**	69.5 / -	30.6 / -	Nd	100.1 / -
10 / 15	31.4 / 5.2	64.5 / 94.5	96.0 / 99.6	**5**	36.8 / 39.7	63.0 / 62.0	Nd	99.8 / 101.7
20	3.1 / -	93.9 / -	96.9 / -	**20 / 15**	4.3 / 6.5	96.5 / 94.7	Nd	100.8 / 101.2
29	5.8 / 6.1	92.3 / 95.1	98.1 / 101.2	**29**	2.7 / nd	94.0 / 102.6	5.5	102.2 / 102.6

By applying first-order reaction kinetics, the rate of hydrolysis of fluazinam for pH 7 and 9 at 25°C and 50°C, as well as the rate of hydrolysis of CAPA at 50 °C was calculated. The experimental data obtained were analyzed by non-linear regression using the program MicroCal Origin (v 3.5). The results of DT_{50} and DT_{90} values are shown at table above.

Table 21: DT50 and DT90 for fluazinam and its metabolite CAPA

	pH 4	pH 7		pH 9	
	50 °C	25 °C	50 °C	25 °C	50 °C
[^{14}C-Phenyl] Fluazinam					
DT_{50} [d]	stable	4.5	0.1	3.5	0.2
DT_{90}		14.8	0.4	11.6	0.6
r^2	-	0.970	0.997	0.997	0.995
[2.6-^{14}C-Pyridine] Fluazinam					
DT_{50}	stable	2.7	0.2	3.9	0.1
DT_{90}		9.1	0.6	13.0	0.3
r^2	-	0.996	0.994	0.998	0.999
CAPA					
DT_{50}	stable	-	31.7	-	7.7
DT_{90}		-	105.3	-	25.7

	pH 4	pH 7		pH 9	
	50 °C	25 °C	50 °C	25 °C	50 °C
r^2	-	-	0.997	-	0.999

Conclusion:
Under acid conditions (pH 4) fluazinam is stable to hydrolysis at 25 °C. Under more neutral and alkaline conditions fluazinam is rapidly hydrolysed with DT_{50} values between 2.7 and 4.5 d (pH 7) and 3.5 to 3.9 d (pH 9) to form metabolite CAPA. At 50 °C CAPA is steadily hydrolysed to DCPA. This degradation product was shown to be stable to hydrolysis. Half lives of CAPA at 50 °C were estimated to be 31.7 d (pH 7) and 7.7 d (pH 9).
Comment (RMS): Study considered acceptable.

Photolysis
Photochemical Degradation of active substance
Reference: Lentz, N.R. and Korsch, B.H. (1995): A Photolysis Study of IKF-1216 (Fluazinam) in Water at pH 5 (Final Report; Document no 5312-94-0119-EF-002) and Lentz, N.R. and Korsch, B.H. (1994): A Photolysis Study of IKF-1216 (Fluazinam) in Water at pH 5 (Part 1, interim report); (Report no. 5312-94-0119-EF-001)
Guideline: U.S. EPA, Subdivision N, 161-2.
GLP: yes, with the exception that the NMR analyses performed at the University of Akron were not done under GLP.
Test item: ^{14}C-IKF-1216-B [^{14}C-Phenyl] Fluazinam, radiochemical purity > 99 %, batch no.: 0571; ^{14}C-IKF-1216-Py [2,6-^{14}C-Pyridine] Fluazinam, radiochemical purity > 97 %, batch no.: 0696
Material and methods:
The direct photolytic degradation of [^{14}C-Phenyl] and [2,6-^{14}C-Pyridine] labelled fluazinam (0.049 µg/mL) was investigated in sterile aqueous buffer solution at pH 5. Test samples were exposed to simulated sunlight (xenon arc light) under 12-hour light/ 12-hour dark cycle for up to 30 days. The temperature was maintained at 25 ± 1 °C during the study. At appropriate sample intervals, light exposed and dark control samples were analysed by radio-HPLC and LSC. Additionally for the identification of degradation products analyses by HPLC, LC/MS and NMR were conducted. Calculations of half life and rate constant were performed with linear regression analyses by the computer program Excel.
Findings:
Results of dark controls: Mean recovery: 93.5 % and 93.7 %. No significant degradation of test substance was noted.

Table 22: Distribution of [14C-Phenyl] Fluazinam and its degradation products (≥ 10 %) expressed as % of AR in light exposed samples

day	IKF-1216	polars	fraction 15-18	G-504	CO_2	recovery
0	96.6	0.2	0.7	0.3	-	98.9
1	63.8	1.7	12.0	9.8	-	95.2
3	36.0	6.9	20.2	15.2	-	89.9
5	14.8	16.7	25.2	16.3	-	85.6
7	8.7	18.3	25.7	14.6	3.0	80.9
10	6.1	21.8	23.9	17.1	3.7	82.5
14	1.9	33.9	20.4	12.4	6.4	83.8
21	1.7	33.3	18.8	9.7	13.0	85.2
28	1.0	38.2	17.4	8.9	17.7	90.4
30	0.9	37.2	17.2	6.4	17.7	85.5

Polars: Multi-component water soluble mixture with different chemical behaviour depending on label position. No individual component accounting for > 10 % AR.

Fraction 15-18: No single component exceeds 10 % of AR.

Table 23: Distribution of [2,6-14C-Pyridine] Fluazinam and its degradation products (≥ 10 %) expressed as % of AR in light exposed samples

day	IKF-1216	polars	fraction 15-18	G-504	CO2	recovery
0	99.0	0.3	0.4	0.3	-	101.3
1	65.0	3.4	12.2	6.2	-	96.4
3	40.0	8.8	20.4	11.6	-	92.1
5	25.6	13.6	23.7	12.9	-	88.7
7	10.6	18.8	25.2	14.0	7.1	88.3
10	6.2	22.9	24.1	12.1	9.3	87.2
14	1.7	30.7	20.2	9.0	12.2	83.5
21	1.6	31.5	19.7	7.9	14.0	83.9
28	0.7	37.9	17.7	4.8	16.0	84.2
30	0.9	37.0	18.8	6.3	16.0	87.1

Polars: Multi-component water soluble mixture with different chemical behaviour depending on label position. No individual component accounting for > 10 % AR.
Fraction 15-18: No single component exceeds 10 % of AR.

Identified minor metabolites: AMPA (max. 4.1 % after 10 days) and HYPA (amounts not stated)
Summary of photolytic degradation steps:

- Reduction and hydrolysis of NO_2, Cl and CF_3 substituents
- Cleavage between phenyl and pyridine ring
- Ring opening leading to complex mixtures of polar compounds
- Oxidative fragmentation with CO_2 production (from both labels)

Table 24: Calculted DT50 and rate constante of [14C-Phenyl] and [2,6-14C-Pyridine] Fluazinam

test substance	DT50 [d]	k [d^{-1}]	r^2
[^{14}C-Phenyl] Fluazinam	2.5	-0.2728	0.977
[2,6-^{14}C-Pyridine] Fluazinam	2.5	-0.2827	0.994

Conclusion:
[^{14}C-Phenyl] and [2,6-^{14}C-Pyridine] labelled fluazinam was rapidly degraded during aqueous photolysis at pH 5 (sterile buffer) and 25 °C. The half life was calculated to be 2.5 d for both labels. Multitude of photolytic degradation products results from a complex degradation pathway with reduction and hydrolysis of NO_2, Cl and CF_3 substituents, the cleavage between the ring systems, ring opening and oxidative fragmentation with CO_2 production. The only major metabolites for both labels are G-504 (max. 17.1 % after 10 days) and CO_2 (max. 17.7 % at day 30).
Comment (RMS):
The recoveries in this study from day 5 onwards are low (81 % – 88 % AR). However, the RMS considers the data sufficient to clarify the metabolic pathway of fluazinam under the influence of light. Thus the study is considered sufficient for further risk assessment.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier

5.1.2.2 Screening tests

Ready biodegradability

Reference: Grützner, I. (2000): Ready Biodegradability of Fluazinam in a Manometric Respirometry Test. Report No. 774898
Guideline: OECD 301F; EU EEC, C.4-D
GLP: yes
Test item: Fluazinam, purity of 98.4%, batch no.: A629/1995
Material and methods:
The ready biodegradability of fluazinam was studied in a "28-Day-Manometric Respirometry Test". 100 mg/L of the test substance was dissolved in test water (purified water and stock solutions of mineral components, adjusted to pH 7.4) and than inoculum (activated sludge from a water treatment plant with a final concentration of 30 mg dry material per litre) was added. Fluazinam was tested in duplicates. Additionally two inoculum controls (without test substance), two procedure controls (with reference substance sodium benzoate), an abiotic (without inoculum and poisoned with mercury dichloride) and a toxicity control (with test and reference substance) were prepared. All test flasks were incubated in the dark for 28 days at 22 °C with continuous stirring. The oxygen consumption, temperature and the pH were recorded at appropriate time intervals. The percent biodegradation of test substance was calculated as ratio of BOD (biochemical oxygen demand of test item) to $ThOD_{NH4\ or\ NO3}$ (theoretical oxygen demand of test item without or with nitrification) x 100.
Findings:
Abiotic control: No significant degradation of test substance.
Toxicity control: After 14 days the biodegradation rate was 63 % based on $ThOD_{NH4}$ and 50 % based on $ThOD_{NO3}$, thus the test substance had no inhibitory effect on activated sludge micro organisms.
Procedure control: After 28 days the biodegradation rate was 95 % based on ThOD.
Inoculum control: After 28 days the BOD in the test flasks was 7 and 18 mg O_2/L (arithmetic mean 12.5 mg O_2/L).
Test substance: After 28 days the BOD in the test flasks was 12 and 14 mg O_2/L (arithmetic mean 13 mg O_2/L). The biodegradation rate was 1 % based on $ThOD_{NH4}$ and 0 % based on $ThOD_{NO3}$. Therefore fluazinam is not ready biodegradable.
Conclusion: Fluazinam is not readily biodegradable under test conditions within 28 days.
Comment (RMS): Study considered acceptable.

5.1.2.3 Simulation tests

Aerobic water/sediment study
Reference: Goodyear, A. (1997): ^{14}C-Fluazinam: Biodegradation in Natural Water-Sediment Systems. Report No. 38/188-1015

Guideline: BBA Guidelines, Part IV, Section 5-1; proposed UK Guidelines for the Conduct of Biodegradability Tests on Pesticides in Natural Sediment-Water Systems (1992)
GLP: yes
Test item: [^{14}C-Phenyl] Fluazinam, radiochemical purity > 98 %, batch no.: 89-48P2; [2,6-^{14}C-Pyridine] Fluazinam, radiochemical purity > 98 %, batch no.: 84-J15P2
Material and methods:
The aerobic aquatic metabolism and degradation of [^{14}C-Phenyl] and [2,6-^{14}C-Pyridine] labelled fluazinam were studied in two water-sediment systems. Approx. 0.032 mg test substance (field application rate ~ 200 g/ha) per test vessels were applied and were incubated at 20°C under aerobic conditions in darkness for up to 100 days.
The sediment with associated water was sampled at two sites in natural environment:
System 1: „Virgina water“, Chatsworth, Derbyshire, UK
System 2: „Emperor Lake“, Windsor Berkshire, UK
The characterisations of both systems are given in table B.8.4.2.2-1.
After sampling a 2.5 cm sediment layer was filled into each incubation vessel (borosilicate glass cylinders with ca. 4.5 cm diameter) and covered with 6 cm depth of associated water. Test vessels were acclimatised to test conditions up to 75 d („Emperor Lake“) and 76 d („Virginia water“). All test units were gently shaken by an orbital shaker and moistened air was passed over water surface. During the acclimatisation period oxygen content and redoxpotential were monitored until test systems were considered as equilibrated. Then the test substance was applied drop wise onto the water surface of each vessels. The effluent air from each incubation unit was passed through a series of traps (one ethanediol trap, one 2 % paraffin in xylene trap, two 0.1 M sodium hydroxide traps) to collect volatile degradation products. One sample per label position was taken for analysis after 0, 6, 24 and 48 hours and 7, 14, 30, 61 and 100 days. The dissolved radioactivity of samples was analysed by HPLC and TLC. Additionally pH, redoxpotential and oxygen content were measured at these sampling times. Non-extractable residues in the sediment (from day 30 and 100 sampling intervals) were characterised by extraction with 0.5 M sodium hydroxide. Further acid hydrolysis and fractionation of soil organic matter into humic- and fulvic acids were performed.

Table 25: Physical and chemical properties of the two test systems:

Parameter	**System 1: „Virginia Water“**	**System 2: „Emperor Lake“**
Water		
Temperature* (below surface) [°C]	9	4.7
pH*	6.9	5.6
O_2-concentration [%]* at surface/5cm above sediment	79.3	96
total hardness [mg/L as $CaCO_3$] **	134/205	71/52
DOC [mg C/L]**	23.7/29.4	16.6/20.3
total nitrogen [mg/L]**	15.4/4.2	<0.1/2.1
total phosphorous [mg/L]**	0.2/0.7	0.1/0.6
Sediment		
pH*	6.6	5.8
C_{org} [%]	3.3	4.3
total nitrogen [%]	0.2	0.2
total phosphorus [mg/kg]	480	560
cation exchange capacity [meq/100 g soil]	9.7	10.0
biomass [µg C/g]**	442/171	371/223

Parameter	System 1: „Virginia Water“	System 2: „Emperor Lake“
Water		
texture (BBA)	slightly loamy sand	medium loamy sandy
particle size distribution:		
sand [%]:	88	75
silt [%]:	5	16
clay [%]:	7	9

* parameter was measured at the time of sampling
** parameter was measured at start and end of the study

Findings:
The two systems did not differ significantly in their texture, C_{org}-content and microbial biomass.
It was stated in the study that the metabolic pathway of phenyl and pyridyl labelled fluazinam was similar, thus results from both treatments were combined and expressed as mean values in table B.8.4.2.2-2. Only degradation products which exceeded 10 % of applied radioactivity are mentioned in the table below. Minor metabolites identified are not mentioned in table below.

Table 26: Radioactivity distribution, partitioning and balance of fluazinam (results in % of applied radioactivity) during the degradation in water- and sediment phase within the “Virginia water” and “Emperor Lake” system

System 1: “Virginia water“ (loamy sand)											
day	**water**	**EXT.R -sed.**	**NER**	**CO_2**	**Total**	**ai water**	**ai sed.**	**AMPA water**	**AMPA sed.**	**xx* water**	**xx* sed**
0	67.3	30.6	1.0	-	99.1	67.0	18.0	nd	7.0		1.4
0.25	69.4	27.1	1.2	nd	99.2	68.9	13.8	nd	7.7		1.3
1	62.5	32.1	2.9	nd	99.5	59.2	12.8	nd	10.7		1.8
2	36.5	50.0	6.4	nd	96.9	34.0	21.5	nd	14.4	2.9	3.8
7	22.2	48.6	16.9	nd	94.6	11.4	6.5	1.4	19.4	2.3	7.2
14	7.6	48.8	30.1	0.2	96.0	0.5	1.7	1.7	21.9	2.6	13.2
30	3.3	37.3	47.2	0.4	94.3	0.2	1.3	0.5	9.9	3.1	15.0
61	3.3	28.9	49.4	1.2	88.5	1.0	7.3	0.4	5.5	1.7	9.4
100	2.0	27.4	55.1	2.0	91.1	-	1.7	-	8.7	0.9	10.7
System 2: “Emperor Lake“ (sandy loam)											
day	**water**	**EXT.R -sed.**	**NER**	**CO_2**	**Total**	**ai water**	**ai sed.**	**AMPA water**	**AMPA sed.**	**xx* water**	**xx* sed**
0	68.5	27.4	2.5	-	98.4	67.7	18.4	nd	4.6		1.1
0.25	65.4	31.1	2.0	nd	98.5	63.9	19.8	nd	5.2		1.8
1	46.8	45.7	4.9	nd	97.5	43.5	28.2	nd	8.1	0.5	2.5
2	42.9	48.5	7.2	nd	98.8	36.4	32.4	nd	7.2	1.8	2.5
7	31.2	43.9	19.0	nd	94.1	20.2	13.8	0.3	15.7	0.6	7.4
14	18.9	42.6	33.5	0.1	95.9	6.2	7.9	0.6	14.3	1.7	11.3
30	14.7	37.9	42.8	0.4	96.1	2.7	13.6	0.4	7.1	3.1	10.5

System 1: "Virginia water" (loamy sand)											
day	**water**	**EXT.R -sed.**	**NER**	**CO_2**	**Total**	**ai water**	**ai sed.**	**AMPA water**	**AMPA sed.**	**xx* water**	**xx* sed**
61	8.5	27.4	55.3	1.7	93.1	0.4	3.9	0.1	6.0	2.3	10.5
100	7.2	21.9	54.3	2.2	85.8	-	2.1	-	2.5	-	12.2

EXT.R-sed: extractable residues in sediment
NER: non extractable residues in sediment
nd: not detected
xx*: total unknowns, mixture of several polar compounds where individual substance did not exceed 2 % AR

Table 27: Maximum concentrations (results in % AR from HPLC analyses) of metabolites HYPA, DAPA, MAPA and AMPA in water and sediment phase within the "Virginia water" (system1) and "Emperor Lake" (system 2)

metabolite	system	Label position	water max [%]	water time [d]	sediment max [%]	sediment time [d]
Minor metabolites						
HYPA	**1**	pyridyl	3.2	7	2.7	7
		phenyl	4.0	7	3.2	14
	2	pyridyl	5.1	14	3.6	30
		phenyl	5.2	7	2.7	30
DAPA	**1**	pyridyl	4.5	7	7.0	7
		phenyl	1.0	7	9.2	7
	2	pyridyl	0.3	30	1.0	100
		phenyl	1.0	14	2.0	14
MAPA	**1**	pyridyl	0.2	7, 14	5.2	2
		phenyl	0.6	14	4.3	7
	2	pyridyl	0.1	14	3.0	7
		phenyl	0.1	14	7.2	7
Major metabolite						
AMPA	**1**	pyridyl	1.9	7	20.2	2
		phenyl	2.5	14	26.7	14
	2	pyridyl	0.9	14	12.7	14
		phenyl	0.4	14	18.9	7

Route of degradation:

Under aerobic aquatic conditions fluazinam was converted to a mixture of at least four metabolites by hydrolysis of the phenyl ring chlorine to a hydroxyl group (HYPA) and reduction of one or both nitro groups (AMPA, MAPA and DAPA). Further degradation products were mainly bound as non-extractable residue to sediment. The mineralization to CO_2 was very low.

Characterisation of NER:

Table 28: Fractionation of NER in sediment, results expressed as mean values of both labels

sample	humin	humic acid	fulvic acid	fulvic acid DCM phase	fulvic acid aqu. phase
system 1, 30 d	35.8	8.9	2.6	0.3	2.4
system 1, 100 d	36.8	1.6	16.7	0.3	16.9
system 2, 30 d	31.1	7.4	4.5	0.5	4.1
system 2, 100 d	36.1	8.6	9.6	1.0	8.3

Half-life calculation:

Table 29: Disappearance times of fluazinam from "Virginia water" and "Emperor Lake" water/sediment systems calculated with Timme & Frehse degradation model (square root 1st order regression)

	water		total system	
system	**DT_{50} [d]**	**DT_{90} [d]**	**DT_{50} [d]**	**DT_{90} [d]**
"Virginia water"	0.8 (r^2: 0.72)	9.2 (r^2: 0.72)	2.9 (r^2: 0.68)	32.1 (r^2: 0.68)
"Emperor lake"	1.2 (r^2: 0.95)	12.7 (r^2: 0.95)	3.2 (r^2: 0.96)	35.4 (r^2: 0.96)

The RMS calculated the degradation rates of the two label positions separately on the basis of single 1st order kinetics. Degradation was calculated starting with the respective highest value observed in the sediments. Formation was not considered. The results are as follows:

Table 30:Degradation rates of the two label positions separately on the basis of single 1st order kinetics**:**

"Virginia" water		phenyl label	pyridyl label	average both labels
	DT_{50}	1.93 d	2.85 d	2.4 d
	DT_{90}	6.41 d	9.47 d	7.9 d
	r^2	0.969	0.986	
"Emperor" water		phenyl label	pyridyl label	
	DT_{50}	1.84 d	4.25 d	3.0 d
	DT_{90}	6.12 d	14.1 d	10.1 d
	r^2	0.942	0.982	
"Virginia" sediment		phenyl label	pyridyl label	
	DT_{50}	2.42 d	3.35 d	2.9 d
	DT_{90}	8.03 d	11.1 d	9.6 d
	r^2	0.969	0.944	
"Emperor" sediment		phenyl label	pyridyl label	
	DT_{50}	6.41 d	9.5 d	7.9 d
	DT_{90}	21.3 d	31.5 d	26.4 d
	r^2	0.76	0.77	
"Virginia" whole system		phenyl label	pyridyl label	
	DT_{50}	3.3 d	2.93 d	3.1 d
	DT_{90}	10.9 d	9.72 d	10.3 d
	r^2	0.996	0.977	
"Emperor" whole system		phenyl label	pyridyl label	
	DT_{50}	5.23 d	6.2 d	5.7 d
	DT_{90}	17.36 d	20.58 d	19.0 d
	r^2	0.956	0.983	
AMPA: "Emperor" sediment		phenyl label	pyridyl label	
	DT_{50}	24.0 d	43.7 d	33.9 d
	DT_{90}	79.8 d	145.2 d	112.5 d
	r^2	0.954	0.906	

Conclusions:

In this study dissipation half lives for fluazinam of about 1 day from the water phase and 3

days from the whole system were calculated by the method of Timme and Frehse. Recalculated half life values (calculated by the RMS) were in the range of 1.84 and 4.25 days for the water phase. For the whole systems recalculated (RMS) single 1st order DT_{50} values were in the range of 2.9 to 6.2 days. Fluazinam was degraded to a mixture of four identified metabolites: HYPA was formed by the hydrolysis of the phenyl ring chlorine to a hydroxyl group. AMPA, MAPA and DAPA were formed by reduction of one or both nitro groups. Only AMPA was reported as major metabolite with amounts of max. 21.9 % AR (i.e. maximum mean of both labels; system 1, day 14) in sediment. Other identified metabolites were found with short peak levels of up to ≤ 8.1 % AR (mean of both labels) and were considered as minor (not relevant) by the RMS. The main dissipation process was the binding of degradation products to non-extractable residue in sediment. The mineralization to CO_2 was very low.

Comments (RMS):

The study offered following deficiencies:

- The two tested sediments (both with coarse texture and high organic carbon content) do not differ significantly from each other in texture, C_{org}-content and microbial biomass. Further they do not represent the "worst case water/sediment system" for dissipation of test substance from water. This case would be represented by a sediment with coarse texture plus low organic carbon content.
- Only one sample per sampling time and test series and label position (^{14}C-phenyl and ^{14}C-pyridyl label) was analysed, recommend are replicates for analysis.
- DT_{50} calculations are based on the method of Timme & Frehse, but data produced by this degradation model are not appropriate as input parameter for FOCUS Surface Water calculations. Square root 1st order regression analyses with only 4 data points were used. Recalculations on the basis of single 1st order kinetics were done by the RMS.

Study was considered acceptable. For details of the selection of input parameters for PEC_{SW} calculations and handling of the shortcomings of the study see chapter B.8.6.1.

5.1.3 Summary and discussion of degradation

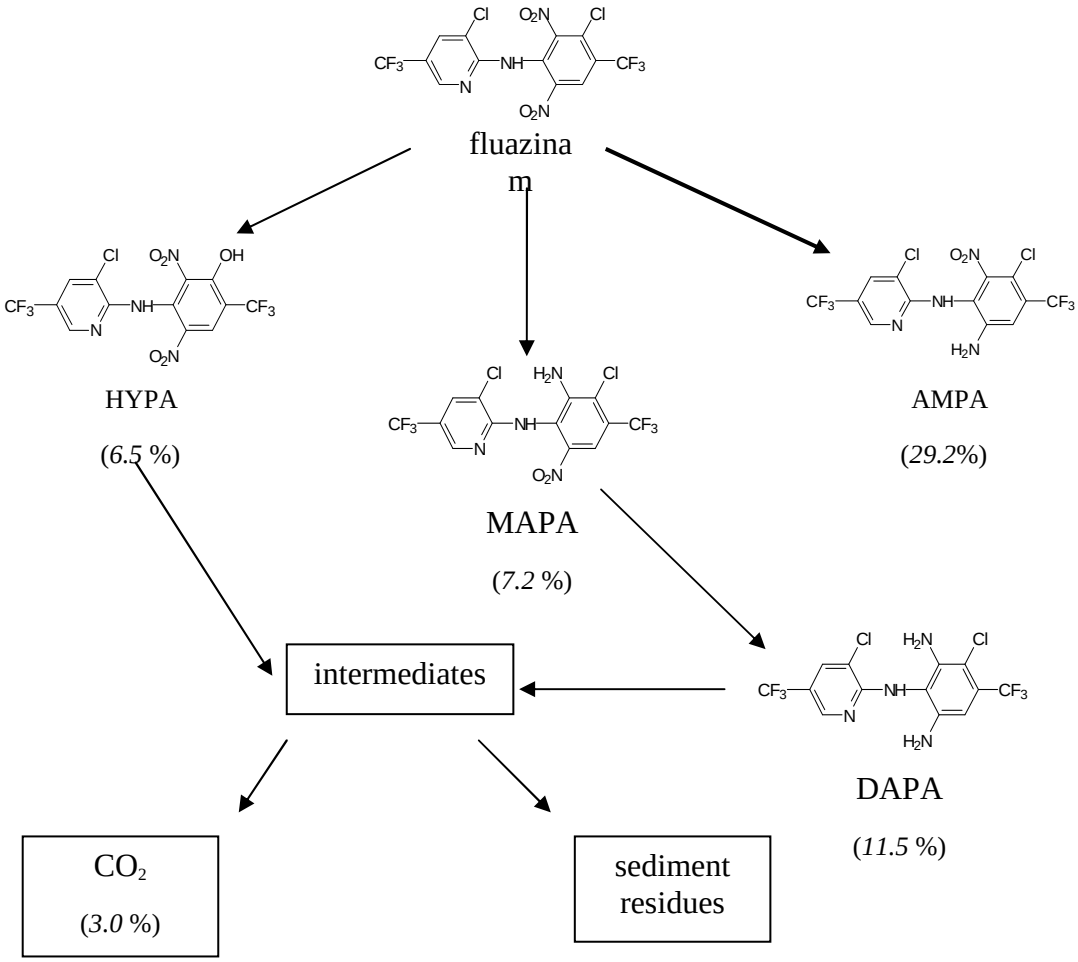
Under acidic conditions fluazinam is stable to **hydrolysis**. Under sterile neutral and alkaline conditions fluazinam is rapidly hydrolysed with DT_{50} values between 2.7 and 4.5 days (pH 7) and 3.5 and 3.9 days (pH 9) to form CAPA. CAPA was identified as major metabolite which accounts for up to 94 % AR at pH 7 and up to 99 % AR at pH 9 (study termination, day 29). Under high temperatures (50 °C) CAPA was shown to metabolise itself to metabolite DCPA. DCPA was shown to be stable to hydrolysis. Half lives of CAPA were estimated to be 31.7 days (pH 7) and 7.7 days (pH 9) at 50 °C.

^{14}C-phenyl labelled and ^{14}C-pyridyl labelled fluazinam degraded rapidly during aqueous **photolysis** at pH 5 (sterile buffer solution) at 25° C. The half life was calculated to be 2.5 days for both labels. The major metabolites are G-504 (max. 17.1 % AR after 10 days) and CO_2 (max. 17.7 % AR after 30 days). A large number of minor degradation products was found, which results from a complex degradation pathway with reduction and hydrolysis of NO_2, Cl and CF_3 substituents, the cleavage between the two ring systems of fluazinam, ring opening leading to complex mixtures of polar compounds, and oxidative fragmentation with CO_2 production.

According to the results of a 28-day Manometric Respirometry test fluazinam is **not readily biodegradable**.

In **water/sediment systems** fluazinam disappeared from the water phase with DT_{50} values (single 1st order kinetics; calculated by the RMS) between 1.93 days (phenyl label) and 2.85 days (pyridyl label) in one system and between 1.84 days (phenyl) and 4.25 days (pyridyl) in the other water/sediment system. The calculated DT_{50} values in the sediment were 2.42 days (phenyl label) and 3.35 days (pyridyl label) in one system and 6.41 (phenyl) and 9.5 days (pyridyl) in the other. With the compartment model ModelMaker DT_{50} values in sediment of 3.0 days and 12.1 days were calculated by the notifier. On the basis of single 1st order kinetics the RMS calculated DT_{50} values for the whole systems between 3.3 days (phenyl label) and 2.9 days (pyridyl label) in one system and between 5.2 days (phenyl) and 6.2 days (pyridyl) in the other system. Residues partitioned rapidly from the water phase into the sediment. Extractable residues in the sediment reached their maxima after 2 days in both systems and amounted for 50 % AR and 48.5 % AR. They declined to 27.4 % and 21.9 % AR after 100 days in the two systems, respectively. At day 0 of incubation 30.6 % and 27.4 % AR were found as extractable residues in the sediment. Non extractable residues in the sediment amounted for up to 55 % AR and 54 % AR at study termination (100 days). Several minor metabolites and a mixture of polar compounds were detected in the water phase and in the sediment. Degradation of fluazinam is via hydrolysis of the phenyl ring chlorine to a hydroxyl group or reduction of one or both nitro groups of the phenyl ring. Identified minor metabolites were HYPA, DAPA and MAPA. AMPA was identified as the major metabolite, which was detected in the sediment in amounts up to 26.7 % AR (phenyl label; day 14) and 20.2 % AR (pyridyl label; day 2) in one system and up to 12.7 % AR (phenyl; day 14) and 18.9 % AR (pyridyl; day 7) in the other system. In the water phase AMPA amounted only up to 2.5 % AR as maximum.

The two water/sediment systems used did not differ significantly from each other with regard to texture, organic carbon content and microbial biomass. As the organic carbon content was rather high in both systems (3.3 % and 4.3 %) a worst case situation with regard to the dissipation of fluazinam from the water phase is not represented. From the batch equilibrium studies it was concluded that a clear correlation between the adsorption of fluazinam to soils (K_f) and the C_{org} content of soils does exist. A statement was submitted by the notifier explaining that due to rapid hydrolysis of fluazinam it is expected that lower organic carbon content of sediments will not effect the dissipation of fluazinam from the water phase significantly.



Proposed metabolic pathway of fluazinam in water/sediment systems
(*values in bracketc: maxima observed in the whole system for a single label position*)

5.2 Environmental distribution

Metabolism

Fluazinam is metabolised by microbial activity. The main metabolic pathway is the formation of bound residues, which were found in amounts of up to 47.2 % of applied radioactivity after 180 days in laboratory studies under **standard conditions**. Metabolites which would indicate cleavage of the bridging amino group were not observed. Mineralization (formation of CO_2) amounted for up to 6 % applied radioactivity after one year under standard conditions. Under aerobic conditions HYPA was the major metabolite which is formed by hydrolysis of the phenyl ring chlorine of fluazinam to a hydroxyl group. The maximum amount found in laboratory studies under standard conditions was 13.9 % AR, after 48 days of incubation. MAPA and DAPA, which are formed by reduction of one or both NO_2 groups, respectively, on the phenyl ring of fluazinam, were found in minor amounts.

Under **anaerobic conditions** MAPA and DAPA were the major metabolites, whereas HYPA was found only in minor amounts. Degradation of fluazinam is accelerated and formation of NER is enhanced under anaerobic conditions but mineralization (CO_2 formation) seems lower.

Degradation

The degradation of ^{14}C-fluazinam (label position on the phenyl or the pyridyl ring) in soil under **aerobic conditions** was investigated in two studies, including two sandy loam soils and one loamy sand. A third study with unlabelled fluazinam included a sandy soil. In the first study the half lives of fluazinam under standard conditions, recalculated separately for the two label positions by the RMS on the basis of single 1st order kinetics, were in the range of 96 and 263 days for the phenyl labelled fluazinam and between 63 and 189 days for the pyridyl labelled fluazinam. The corresponding DT_{90} values were in the range of 320 – 873 days and 210 – 628 days, respectively. In the second study a DT_{50} value of 17 days was calculated for a mixture of the two label positions. In the third study the calculated DT_{50} value for unlabelled fluazinam was 62 days (single 1st order kinetics).

The data derived from the test at 10° C were not sufficient to calculated reliable degradation rates. However, it was possible to conclude from the data available that **low temperatures** as well as exaggerated application rate reduced the metabolism of fluazinam.

Under **anaerobic conditions** degradation of fluazinam is fast. Flooding the soil on day 0 of incubation yielded DT_{50} values of 3.8 days, for both, the phenyl labelled and the pyridyl labelled fluazinam. DT_{90} values were 12.6 and 12.8 days for the two label positions, respectively.

Degradation rates for the main soil metabolite HYPA were investigated in one study including three different soils. Calculated DT_{50} and DT_{90} values were in the range of 54 to 148 days (arithm. mean: 95 d) and 179 to 490 days (arithm. mean: 305 d), respectively (single 1st order kinetics).

Photolysis

Under the influence of light degradation of fluazinam on soil is significantly increased. Degradation rates were recalculated by the RMS on the basis of single 1st order kinetics for the two label positions separately. The DT_{50} values for the phenyl ring label were 72 days (dark control) versus 22 days (light condition) and for the pyridyl label 68 days (dark) versus 17 days (light). The corresponding DT_{90} values were 238 days (phenyl label) and

226 days (pyridyl label) for the dark control and 72 days (phenyl label) and 65 days (pyridyl label) for the light exposed samples. The light intensity was comparable to southern European conditions. Under both, light and dark conditions conversion to bound residues was the main pathway. Conversion to bound residues was more extensive for the light-exposed samples. In general, photolysis appears to accelerate reactions that occur in soil under dark conditions. The presence of HYPA at comparable levels in the dark controls and the light samples suggests it is a product of soil metabolism. AMPA, however, is found in the light-exposed samples at levels slightly higher than in the dark controls (5 % AR versus <1 % AR).

Field studies

Two European field studies and four field studies conducted in the USA were submitted. The risk assessment of fluazinam is based on the data from the European field studies. The studies from the USA were not considered relevant by the RMS.

Two soil degradation trials were carried out with fluazinam (formulation Shirlan 50 SC) in the UK over a period of 15 and 16 months. The test substance was applied either on potatoes or on bare soil on ten occasions (7 – 10 day interval) at a rate of 0.3 kg ai/ha each. Fluazinam residues in the upper soil layer (10 cm) taken from bare ground plots declined throughout the trial at both sites from initial residues of 0.61 mg/kg and 0.38 mg/kg to 0.02 mg/kg and 0.01 mg/kg after 370 days. Concentrations in soil from the planted plots were significantly lower. No quantifiable residues of fluazinam (LOQ = 0.01 mg/kg) were found in deeper soil layers with only very few exceptions. Concentrations of soil metabolite HYPA were in the range of 0.03 and 0.09 mg/kg in the upper soil layer. No measurable amounts of HYPA were found in all the 10 – 20 cm soil samples.

Four soil degradation trials were carried out with fluazinam (50 % w/v SC formulation) in Germany over a period of 12 months. The test substance was applied on bare ground at a rate of 1.35 kg ai/ha. Initial concentrations of fluazinam in the upper soil layer were in the range of 0.7 to 1.0 mg/kg soil, which declined to <0.01 to 0.08 mg/kg after 306 days. In deeper soil layers no measurable residues of fluazinam were found. The residues of the metabolite HYPA were below the LOD (= 0.01 mg/kg) in the upper soil layer, with very few exceptions. HYPA was not detected in deeper soil layers.

The calculated dissipation rates of fluazinam under field conditions (UK and Germany) were in the range of 8.3 and 40.8 days (single 1st order kinetics), with a geometric mean of 20.4 days. For FOCUS ground water modelling temperature normalised (20 °C) DT_{50} values were used. These were in the range of 8.4 and 25 .7 days, with a geometric mean of 16.4 days.

5.2.1 Adsorption/Desorption

Adsorption and leaching behaviour

Fluazinam showed low mobility in a batch equilibrium study with four different soils. The calculated K_{OC} values were in the range of 1 705 to 2 316 mL/g, with an arithmetic mean of 1958 mL/g. The results obtained indicate that a large percentage of fluazinam is irreversibly adsorbed onto soils with different properties. Increasing adsorption (K_f) was observed with increasing organic matter content. For soil metabolite HYPA the calculated K_{OC} values from a study conducted with six different soils, were in the range of 450 and 1 700 mL/g, with an arithmetic mean of 920 mL/g. From this batch equilibrium study medium to low mobility for HYPA can be concluded. In acidic soils higher K_{OC} values were observed compared to alkaline soils. When excluding the two acidic soils, which are

considered not representative for potato growing areas by the RMS the arithmetic mean K_{OC} value for the four remaining soils is 630 mL7g.
According to the results of a column leaching study it is unlikely that normal agricultural use of fluazinam will result in significant contamination of ground water. After application of fluazinam at a rate equivalent to 750 g ai/ha on sand, loamy sand and sandy loam soils, less than 2 % of the applied amount leached through the soil columns.

5.2.2 Volatilisation

According to the phys./chem. parameters of fluazinam this substance is expected to have medium to high potential for volatilisation. With a vapour pressure of $7.5 \pm 0.8 \times 10^{-3}$ Pa (20° C), a water solubility of 0.135 mg/L and a resulting Henry's law constant of 25.9 Pa x m^3 x mol^{-1} (20°C) fluazinam has a rather high potential for being available in air. On the basis of the available data it can be concluded that the half-life of fluazinam by photochemical oxidative degradation in air most probably is > 2 days. Absorption of light above 290 nm was shown for fluazinam with a molar extinction coefficient (**ε**) > 10. The quantum yield (Φ) of fluazinam was stated to be 1.7×10^{-5} mole/Einstein (pH 6 distilled water).
Hydrolytic (pH 7 and 9; 25 °C) and aqueous photolytic degradation of fluazinam was observed, with DT_{50} values <4 days.
For the time being no harmonised model/method to calculate concentrations in air is available.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 31: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient 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 Acceptable	Sanders, J. (1992) (Document 4039-91-0386-AS-001)
Partition coefficient 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)
Bioconcentration EPA Guideline 165-4	BCF phenyl label 1090* / phyridyl label 960* * based on total ^{14}C residues	Acceptable	Lentz, N. R. & Huhtanen, K. L. (1994) Report No. 5311-93-0013-EF-001

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Not necessary because measured bioaccumulation data are available

5.3.1.2 Measured bioaccumulation data

Reference: Lentz, N. R. & Huhtanen, K. L. (1994): Uptake, Depuration, and Bioconcentration and Metabolism of (Fluazinam) Carbon-^{14} IKF-1216 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow Through Test Conditions. Report No. 5311-93-0013-EF-001
Test guideline: EPA Guideline 165-4
GLP: Yes
Test item: ^{14}C-phenyl labelled Fluazinam (radiochemical purity > 98 %) and ^{14}C-pyridyl labelled Fluazinam (radiochemical purity > 98 %), Lot Numbers; T9002 and 0201
Material and methods:
Bluegill sunfish were exposed to ^{14}C-phenyl and ^{14}C-pyridyl labelled Fluazinam under flow-through conditions to assess the uptake, the depuration, the bioconcentration and metabolism of the active substance. For the 35-day exposure period mean measured water concentrations of 0.66 (±0.176) µg/L for the ^{14}C-phenyl label and 0.77 (±0.124) µg/L for the ^{14}C-pyridyl label were maintained. Observations of mortality and sublethal effects were made twice daily. After the exposure fish were placed in clean water for up to 21 days (depuration period). During the uptake and depuration phase radioanalyses (LSC) of fillet (edible portion), whole fish, viscera (non edible portion) and water samples were performed. Additionally HPLC analyses were performed for fish samples to evaluate the ^{14}C-distribution in tissues, the extraction-partitioning behaviour and the identification of metabolites. The BCF_{ss} was calculated as the ratio of concentration in fish (C_f) and in the water (C_w). Additionally the kinetic bioconcentration factor (BCF_k) at steady state as the

ratio of the rate constants of uptake (k1) and depuration (k2) was determined. For the calculation of rate constants the BIOFAC computer program was used. Water quality parameters like temperature, dissolved oxygen and pH were recorded initially and at fixed intervals during the study. The fluazinam concentration in the water phase was also measured at three time points: 21, 28 and 35 days uptake phase.

Findings:

The fluazinam concentration (both labels) in water phase ranged between 0.591 and 0.862 µg/L, which corresponds to 56 – 70 % of the total radioactive residues (TRR).

Table 32: Results of bioconcentration in bluegill sunfish after 35 days exposure to phenyl and pyridyl labelled fluazinam.

phenyl label: C_w = 0.66 µg/L	whole fish	viscera	fillet
Total ^{14}C tissue residues after 35 d [µg/kg]	720	1100	230
BCFss	1090	1670	348
k_1 [1/d]	117 ± 8	-	-
k_2[1/d]	0.11 ± 0.01	-	-
BCF_k	1018 ± 96	-	-
CT_{50}	6.0 ± 0.4 d	-	-
Time to reach 90 % steady state	20 ± 1 d	-	-
Elimination during 14 d (21 d) depuration	78 % (78 %)		

pyridyl label: C_w = 0.77 µg/L	whole fish	viscera	fillet
Total ^{14}C tissue residues after 35 d [µg/kg]	740	910	210
BCFss	960	1180	273
k_1 [1/d]	114 ± 5.1	-	-
k_2[1/d]	0.14 ± 0.01	-	-
BCF_k	827 ± 60	-	-
CT_{50} [d]	5.0 ± 0.3	-	-
Time to reach 90 % steady state [d]	17 ± 1	-	-
Elimination during 14 d (21 d) depuration	76 % (79 %)		

Analysis of residues (TRR) in tissues:

After the extraction with acetonitrile, hexane and acetonitrile:water the majority of extractable ^{14}C-residues was found in the acetonitrile fraction, for an average of 32.5 % TRR in fillet and 37.5% TRR in viscera. In hexane extracts an average of 6.7 %TRR (fillet) and 9.3 % TRR (viscera), and in acetonitrile:water extracts an average of 12.8 %TRR (fillet) and 9.3 % TRR (viscera) were analysed. Additionally in PES (postextraction solids) an average of 48 %TRR (fillet) and 29 %TRR (viscera) were found.

Table 33: Identified metabolites in fish fillet (acetonitrile extracts)

Compound	**28 days exposure**		**35 days exposure**	
	phenyl-label (mg/kg)	**pyridyl-label (mg/kg)**	**phenyl-label (mg/kg)**	**pyridyl-label (mg/kg)**
Fluazinam	ND	ND	NQ	NQ
AMPA	ND	0.019	0.009	0.012
MAPA	ND	0.006	NQ	0.001
DAPA	ND	ND	ND	0.002
unknown metabolite	0.018	0.011	0.003	0.006
Total Residue	0.199	0.209	0.232	0.224
Total metabolites [%TRR]	9.0 %	**17.2 %**	5.2 %	9.4 %

ND = not detected; NQ = not quantifiable

Table 34: Identified metabolites in fish viscera (acetonirile extracts)

Compound	28 days exposure		35 days exposure	
	phenyl-label (mg/kg)	pyridyl-label (mg/kg)	phenyl-label (mg/kg)	pyridyl-label (mg/kg)
Fluazinam	0.021	0.008	0.007	0.010
AMPA	0.008	0.042	0.030	0.048
MAPA	ND	ND	0.007	0.018
DAPA	ND	ND	ND	0.006
unknown metabolite	0.032	0.047	0.024	0.030
Total Residue	1.226	1.193	1.122	0.966
Total metabolites [%TRR]	5.0 %	8.1 %	6.1 %	**11.6 %**

ND = not detected

Metabolism: The patterns of ^{14}C-residues obtained by HPLC analyses of both label positions were very similar, thus it can be concluded that in fish to a certain degree no cleavage of the amine linkage between the two ring system of fluazinam occurred. The ^{14}C-residues which were identified included fluazinam, AMPA, MAPA and DAPA. Each of the residues of total metabolites were accounted for max. 17.2 % of the fillet after 35 days and max. 11.6 % of the viscera after 28 days. Additionally numerous other ^{14}C-components were presented but none of the single compounds was found in amounts ≥ 10 %.

Conclusion:

Fluazinam accumulated in whole fish with BCF of values of 960 and 1090. In non-edible portions BCF values of 1670 and 1180 were determined. All BCF values are based on calculations with total ^{14}C-residues. The 90 % level of steady state was reached after 17 – 20 days. During the depuration period the ^{14}C-residues were incompletely eliminated after 14 days and 22 and 24 % of the TRR remained in the whole fish. The depuration half-life (CT_{50}) was estimated to be 5 – 6 days. In general the high BCF of 960 – 1090 (whole fish) and the incomplete elimination of radioactive residues (22 – 24 % remained in fish after 14 days) indicate a potential to bioaccumulation.

Comment (RMS): Study considered acceptable.

5.3.2 Summary and discussion of aquatic bioaccumulation

The log P_{OW} of Fluazinam is 4.03. In a bioaccumulation study Fluazinam accumulated in whole fish with BCF values of 960 and 1090. In non-edible portions BCF values of 1670 and 1180 were determined.

5.4 Aquatic toxicity

Table 35: Summary of relevant information on aquatic toxicity

Method	Results							Remarks	Reference
	test organism	test condition	time	endpoint	test conc.	NOEC (µg ai/l)	LC/EC50 (µg ai/l)		
FIFRA Guideline 72-1	*Oncorhynchus mykiss* Rainbow trout	flow through	96 hr	mortality	m	15	36		Gelin & Laveglia 1992
US EPA § 72-1	*Oncorhynchus mykiss* Rainbow trout	flow through	96 hr	mortality	m	≤ 57	110		Hill 1985
FIFRA Guideline 72-1	*Lepomis macrochirus* Bluegill sunfish	flow through	96 hr	mortality	m	21	55		Gelin & Laveglia 1993
OECD 203	*Brachydanio rerio* Zebra fish	flow through	96 hr	mortality	m	19	89		Peither 2001a
OECD 203	*Poecilia reticulata* Guppy	flow through	96 hr	mortality	m	22	109		Peither 2001b
FIFRA Guideline 72-3	*Cyprinodon variegatus* Sheepshead minnnow	flow through	96 hr	mortality	m	80	120		Shults et al 1993
OECD 202	*Daphnia magna* Waterflea	flow through	48 hr	immobility	m	54	220		Shults et al 1992
OECD 201	*Pseudokirchn. subcapitata* Green alga	static	96 hr	biomass growth rate	m	48	160 > 220		Smyth & Tapp 1987
ASTM 1991, EPA OPPTS 850.4400	*Lemna gibba* Duckweed	static renewal	7 d	biomass growth rate	im	35.9	> 69.1	additional information	Boeri & Ward 2001

Method	Results							Remarks	Reference
	test organism	test condition	time	endpoint	test conc.	NOEC (µg ai/l)	LOEC (µg ai/l)		
OECD 204	*Oncorhynchus mykiss* Rainbow trout	flow through	28 d	mortality weight	m	12	24		Sankey et al 1992
FIFRA Guideline 72-4	*Pimephales promelas* Fathead minnow (ELS)	flow through	34 d 34 d 4 d	survival growth hatchability	m	5.3 5.3 10	10 10 23		Fillmore & Laveglia 1993
FIFRA Guideline 72-5	*Pimephales promelas* Fathead minnow (Life cycle)	flow through	5 d >161 d 278 d 30 d 5 d	F_0 hatchability F_0 reproduction F_0 growth F_1 survival F_1 hatchability	m	6.4 2.9 2.9 6.4 6.4	14 6.4 6.4 14 14		Shults et al. 1995
OECD 202	*Daphnia magna* Waterflea	static renewal	21 d	mortality reproduction growth	n	50 50 12.5	100 100 25		van den Bogaaert et al. 1991
FIFRA 72-4	*Daphnia magna* Waterflea	flow through	21 d	mortality reproduction growth	m	68	140		Shults et al. 1995
Proposed BBA Guideline 1995	*Chironomus riparius* Midge	static	26 d	emergence	in	6.25	12.5		Stewart & Shillabeer 1997

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference: Gelin, M.D & J. Laveglia (1992): Technical Fluazinam (IKF-1216) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Report No. 5099-91-0422-TX-002
Test guideline: FIFRA Guideline 72-1
GLP: yes
Test item: Fluazinam techn.: 96.8 % w/w, lot no. 1030/91
Material and methods:
A 96 hours acute toxicity test of fluazinam to rainbow trout was performed. 20 fish (10 per replicate) were exposed to nominal test concentrations of 0 (dilution water control), 0 (solvent control, acetone), 19, 27, 39, 56 and 80 µg/L, respectively, under flow through conditions. The fish were 5.1 cm (mean) in length and had an average weight of 2.0 g. Fish were exposed to test concentrations and controls under the following conditions: 16/8-hour light/dark photoperiod, 12 – 13 °C, pH 6.8 – 7.1, 68 – 98 % O_2 saturation, a total hardness of 30 mg/L as Ca CO_3 and a specific conductivity of 120 µmhos/cm. Analyses of test substance were conducted at the start and end of the test.
Findings:
Mean measured concentrations were 10, 15, 28, 33 and 56 µg/L, therefore the assessment is based on mean measured concentrations.
Behavioural or sublethal effects like changing of pigmentation (darkening), partial and complete loss of equilibrium and lethargy were observed at test concentrations of 28 and 33 µg/L, therefore the 96 hours "no effect" concentration (NOEC) was determined to be 15 µg/L. After 96 hours at 33 µg/L 35 % and at 56 µg/L 100 % mortality was noted. The 96 hours LC_{50} was estimated to be 36 µg/L (95% CL 33 – 56 µg/L).
Conclusion: LC_{50} (96 h): 36 µg/L and NOEC: 15 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Hill, R. W. (1985): PP192: Determination of Acute Toxicity to Rainbow Trout (*Salmo gairdneri*). Report No. BL/B/2560
Test guideline: US EPA § 72-1
GLP: yes
Test item: PP192 (technical fluazinam): 97.3% w/w, Lot no. 8303-4
Material and methods:
The acute toxicity of fluazinam to *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) was studied in a 96 hours flow-through test. 20 fish per treatment (with a mean length of 34.6 mm and a mean weight of 0.54 g) were exposed to test concentrations of 0.056, 0.075, 0.1, 0.18, 0.32, 0.56 mg/L, one solvent control (acetone and Tween 80) and one dilution water control. For chemical analysis of test substance samples were taken daily. During the study the following physical parameters were monitored in fish exposure vessels: 10.6 – 11.4 mg/L O_2, pH 7.6 – 7.8, 11.8 – 12.7 °C, total hardness 50 – 56 mg/l as $CaCO_3$ and conductivity 130 – 170 µS/cm.
Findings:
Mean measured concentrations of fluazinam were 0.057, 0.064, 0.091, 0.16, 0.27 and 0.46 mg/L (82.1 – 101.8 % of nominal concentrations), thus toxicity endpoints are based on mean measured concentrations. After 93 hours at all tested concentration behavioural or sublethal effects (loss of equilibrium, darkening in pigmentation, surfacing and rapid respiration) were observed, therefore

the NOEC for sublethal effects was < 0.057 mg/L. Mortalities were observed at concentrations ≥ 0.091 mg/L. The 96 hours LC_{50} was calculated to be 0.11 mg/L (95 % CL 0.1 – 0.13 mg/L).
Conclusion: LC_{50} (96 h): 110 µg/L and NOEC ≤ 57 µg/L, based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Gelin, M.D. & J. Laveglia (1993): Technical Fluazinam (IKF-1216) – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. Report No. 5099-91-0421-TX-002
Test guideline: FIFRA Guideline 72-1
GLP: yes
Test item: Fluazinam techn.: 96.8 % w/w, lot no. 1030/91
Material and methods:
A 96 hours test on the acute toxicity of fluazinam to bluegill sunfish was performed under flow through conditions at five nominal test concentrations, one control and one solvent control (acetone). The nominal test concentrations were 31, 45, 64, 91 and 130 µg/L, respectively. Twenty fish (10 per replicate, fish had a mean length and weight of 36 mm and 1.1g) were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, temperature was maintained at 21 °C, pH 6.7 – 7.1, 76 – 102 % O_2 saturation and total alkalinity 20 – 24 mg/l $CaCO_3$.
Findings:
Mean measured exposure concentrations were 21, 34, 44, 66 and 93 µg/l, respectively. All toxicity endpoints are based on mean measured concentrations.
No mortalities and sublethal effects were observed in controls and at the lowest concentration of 21 µg/L, thus the NOEC was 21 µg/L. Behavioural or sublethal effects (loss of equilibrium, lethargy, and swimming at the surface) were noted at 66 µg/L. After 96 hours 10 % mortality was observed at 34 µg/L and at the highest concentration of 93 µg/l all fish were dead. The 96 hours EC_{50} was calculated to be 55 µg/L (95% CL 44 – 66 µg/L).
Conclusion: LC_{50} (96 h): 55 µg/L and NOEC: 21 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Peither, A. (2001a): Acute Toxicity of Fluazinam to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Flow-Through Test. Report No. 813431
Test guideline: OECD 203
GLP: yes
Test item: Fluazinam tech.: 98.4 % w/w, batch no.: A629/1995
Material and methods:
The acute toxicity of fluazinam to zebra fish was assessed in a 96 hours test under flow-through conditions. Seven fish were exposed in replicates to 11, 25, 52, 110, 250 µg/L, one dilution water control and one solvent control (N,N-dimethylformamide). The following exposure conditions were measured during test period: pH 7.8 – 8.2, 22 – 23 °C, a total hardness of 216 mg/L as $CaCO_3$, 7.2 – 8.2 mg/L dissolved O_2 and a light intensity of 50 – 500 Lux (16/8 hours light/dark photoperiod). After 24 hours in concentrations ≥ 52 µg/L the test item was noted at the surface of water. The body weight and length of ten fish were measured at the start of the test: fish had an average weight of 0.18 ± 0.04 g and a mean length of 2.8 ± 0.2 cm. For the analysis of test concentrations, duplicate samples were taken at the start of the test, after 48 hours and at the end of the test.
Findings:
Mean measured concentrations were: not analysed, 19, 49, 79 and 208 µg/L, all reported results are based on mean measured concentrations. After 96 hours no mortalities or other symptoms of

intoxication were noted in controls and concentration up to 19 µg/L. Thus the NOEC was 19 µg/L. After 72 hours at the next higher concentration level (49 µg/L) mortalities and sublethal effects (fish mainly at water surface) were observed. At the highest concentration level all fish died until 48 hours. The 96 hours EC_{50} was calculated to be 89 µg/L (95% CL 64 – 123 µg/L).
Conclusion: LC_{50} (96 h): 98 µg/L and NOEC: 19 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Peither, A. (2001b): Acute Toxicity of Fluazinam to Guppy (*Poecilia reticulata*) in a 96-Hour Flow-Through Test. Report No. 813453
Test guideline: OECD 203
GLP: yes
Test item: Fluazinam tech.: 98.4 % w/w, batch no.: A629/1995
Material and methods:
A 96 hours test on the acute toxicity of fluazinam to guppy, was performed under flow through conditions at five nominal test concentrations, one dilution water control and one solvent control (N,N-dimethylformamide). The nominal test concentrations were 2.4, 7.6, 24, 78, and 250 µg/L, respectively. The fish were 3.7 ± 0.3 cm (mean) in length and had an average weight of 0.48 ± 0.21 g (measured at start of the test from 10 fish). Two replicates with seven fish each were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, 22 – 23 °C, pH 7.7 – 8.0, ≥ 7.3 mg/L dissolved O_2 and a total hardness of 198 mg/l as $CaCO_3$. Chemical analyses of the test item were performed at concentrations ≥ 24 µg/L and samples were taken at the start of the test (0 h), after 48 hours and at the end of the test (96 h).
Findings:
Mean measured concentrations were: not analysed, not analysed, 22, 68 and 234 µg/L. The reported results are related to mean measured concentrations. No mortalities and other symptoms of intoxication were observed at concentrations up to 22 µg/L, therefore the NOEC was determined to be 22 µg/L. After 48 hours mortalities and effects (staying at the bottom of the test vessels) were noted at 68 µg/L. At the highest concentration (234 µg/L) after 48 hours 100 % mortality was recorded. The 96 hours LC_{50} was calculated to be 109 µg/L (95% CL 52 – 226 µg/L).
Conclusion: LC_{50} (96 h): 109 µg/L and NOEC: 22 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Reference: Shults, S. K, A. W. Brock & L. Laveglia (1993): Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5017-91-0415-TX-002
Test guideline: FIFRA Guideline 72-3
GLP:
Test item: Fluazinam techn.: 100 % ai, lot # 1030/91
Material and methods:
A 96 hours test on the acute toxicity of fluazinam to marine fish (*Cyprinodon variegatus*), was performed under flow-through conditions at five nominal test concentrations, one dilution water control and one solvent control (acetone). The nominal test concentrations were 0.13, 0.22, 0.36, 0.6, and 1.0 mg/L, respectively. A representative sample of fish were measured (N = 30) and fish had a mean length of 26 (24 – 35) mm and an average weight of 0.41 (0.25 – 0.7) g. Twenty organisms (ten per replicate) were exposed to each test concentration under the following test conditions: 16/8-hour light/dark photoperiod, 22 – 23 °C, pH 7.8 – 8.2, 64 – 94 % oxygen

saturation and a salinity of 31 – 32 ‰. Chemical analysis of test item concentrations in test media was carried out at 0, 48 and 96 hours of the exposure period.
Findings:
Mean measured concentrations were 0.08, 0.14, 0.24, 0.33 and 0.52 mg/L. No effects were observed at control and lowest concentration (0.08 mg/L) tested. Therefore the NOEC was 0.08 mg/L and the LOEC 0.14 mg/L. At 0.24 mg/L after 24 hours the mortality was 100 %. The 96 hours LC_{50} was calculated to be 0.12 mg/L (95% CL 0.08 – 0.24 mg/L).
Conclusion: LC_{50} (96 h): 120 µg/L and NOEC: 80 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

5.4.1.2 Long-term toxicity to fish

Prolonged toxicity (21 day exposure) to fish (IIA 8.2.2.1)
Reference: Sankey, S. A., Tapp, J. F., Caunter, J. E. & Stanley, R. D. 1992 Fluazinam: The 28 Day LC50 to Rainbow Trout (*Oncorhynchus mykiss*). Report No: BL4167/B
Test guideline: OECD 204
GLP: yes
Test item: Fluazinam techn., purity: 98.1 %, batch no: not stated
Material and methods:
The prolonged toxicity of fluazinam to rainbow trout (*Oncorhynchus mykiss*) was assessed under flow through conditions over a 28 day exposure period. Fish were exposed to five nominal concentrations: 5.6, 10, 18, 32 and 56 µg/L, a dilution water control and a solvent control (DMF). Ten trout per treatment and control were incubated under a 16/8-hour light/dark photoperiod and were fed daily during the study. Environmental test conditions were determined daily for the first three days and then 3 times per week, mean values were 15.0 – 15.3°C, pH 7.5 – 7.86, 8.6 – 10.0 mg/L O_2 content, a conductivity of 176 – 207 µS/cm and a dilution flow-rate of 240 – 255 mL/min. The total hardness was determined by titration and was 40.3 mg/L as $CaCO_3$.
The mortality was recorded daily, behaviour and appearance of fish were checked on days 4, 7, 10, 14, 21 and 28 in each test vessel. At the end of the exposure period the length and weight of alive fish were measured. Chemical analyses of fluazinam were conducted on day 1, 2, 3, 8, 10, 13, 17, 20, 23 and 28 at each tested concentration.
Findings:
Mean measured concentrations were 4.0, 7.4, 12, 24 and 44 µg/L, all endpoints are based on mean measured concentrations. During the 28 days exposure period no sublethal effects and no mortalities were noted in the dilution water control and in concentrations up to 12 µg/L. At day 28 30 % of fish were dead at 24 µg/L and 100 % at 44 µg/L. At this two highest concentrations sublethal effects like reduced or no feeding, dark discoloration, quiescence, surfacing and rapid respiration were also observed. Additionally the growth (mean length and weight) was effected at concentrations of 24 and 44 µg/L. Thus the 28 days NOEC was 12 µg/L and the LC50 was calculated to be 26 µg/l (95 % CL 21 – 32 µg/L)
Conclusion: 28 d LC_{50} (mortality): 26 µg/L, 28 d NOEC and LOEC (mortality, sublethal effects, growth): 12 µg/L and 24 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Fish early life stage toxicity test (IIA 8.2.2.2)
Reference: Fillmore, G. E. & J. Laveglia (1993): Technical Fluazinam (IKF-1216) – The Toxicity to Fathead Minnow (*Pimephales promelas*) During Early Life-Stage Exposure. Report No: 5018-91-0425-TX-002
Test guideline: FIFRA Guideline 72-4
GLP: Yes

Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91
Material and methods:
The chronic effects of fluazinam to early life stages of fathead minnow were performed in flow through exposure systems. Organisms (eggs and fry) were exposed to nominal concentrations of 1.6, 3.1, 6.3, 12 and 25 µg/L, a dilution control and a solvent control (DMF). At test initiation 2 x 60 eggs (≤ 24 hours old) per treatment and control were incubated in egg incubation cups for up to 4 days (hatch period), after hatching 2 x 40 fry per treatment and control were transferred into exposure aquaria and exposed for up to 30 days (posthatch period). Fry were fed with live brine shrimp nauplii three times daily (weekday) or two times daily (weekend). The following environmental test conditions were maintained: Dissolved oxygen: 7.9 – 8.6 mg O_2/L, pH 6.8 – 7.2, a total hardness of 25 – 26 mg $CaCO_3$/L, a specific conductivity of 140 µmhos/cm and a 16/8-hour light/dark photoperiod.
Observations for mortality and abnormal appearance or behaviour were made daily until complete swim up. At study termination weight and length were determined. The following endpoints were assessed: organism survival at hatch, larval survival and larval growth (wet weight and total length).
Samples for chemical analyses of fluazinam in test solutions were removed from both replicates of each tested concentration and the control on day 0, 5, 12, 19, 26 and 34.
Findings:
Mean measured exposure concentrations were 1.6, 2.7, 5.3, 10 and 23 µg/l, all endpoints are based on mean measured concentrations.
The effects in dilution and solvent control did not significantly differ, therefore controls were pooled for statistical analysis. After the hatching period (day 4) survival was significantly effected at 23 µg/L (50 % mortality). At test termination significant effects on larval survival were already observed at 10 µg/L (30 % mortality). The growth was not influenced in the control and all treatment levels up to 5.3 µg/L at the end of testing. The larval survival was significantly effected at the two highest concentration levels and these treatments were excluded from statistical analysis of growth. Based on these data the 34 d NOEC for survival of larvae and growth was 5.3 µg/L and the 4d NOEC for survival at hatching was 10 µg/L. The 34 d LOEC for survival of larvae and growth was 10 µg/L and the 4d LOEC for survival at hatching was 23 µg/L.
Conclusion: Survival and growth: 34 d NOEC = 5.3 µg/L, LOEC = 10 µg/L; hatchability: 4 d NOEC = 10 µg/L, LOEC = 23 µg/L
Comment (RMS): Study considered acceptable.

Fish life cycle test (IIA 8.2.2.3)
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. Report No:5107-92-0035-TX-00
Test guideline: FIFRA Guideline 72-5
GLP: Yes
Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91
Material and methods:
The chronic effects of fluazinam to fathead minnow (*Pimephales promelas*) were studied for a complete life-cycle over 278 days. Additionally the progeny (F_1) was exposed for 30 days post hatch. The following endpoints were observed during the study: Hatching success, survival, growth (wet weight and body length) of first generation fish (F_0) and hatching success survival, growth (wet weight and body length) of their progeny (F_1).
The organisms were exposed to five nominal concentrations (1.3, 2.5, 5.0, 10 and 20 mg/L), a dilution control and a solvent control under flow-through conditions.

The exposure system was a two-tiered system, consisting of an upper and a lower level waterbath. Each waterbath contained fourteen exposure aquaria. The exposure of embryos started in aquaria in the upper level water bath and 100 embryos (2 x 50) were exposed in egg incubation cups to each treatment and control for up to 5 days. After 5 days the hatching success was calculated based on the number of introduced embryos. Furthermore 50 (2 x 25) newly hatched larvae were selected for each tested concentration and controls, and transferred in larval growth chambers. These chambers were examined daily for dead larvae. After 30 and 61 days each larval group was photographed over a grid to determine total length. Additionally, percent larval survival was also noted. At day 37 (post hatch) fish were released from growth chambers to the corresponding aquarium and after 61 days (post-hatch) 25 larvae were randomly selected to remain in each exposure vessels. On day 151 all fish were examined to confirm the existence of reproductive males and females to isolate spawning groups.
On day 161 one male and two females (representing one spawning group) were transferred to spawning aquarium in the second lower level water bath. Remaining fish were also continued in exposure. Dead males in spawning groups were replaced by males from this remaining fish. Females were not replaced. Observations for the presence of eggs were made daily. 2 x 50 embryos from the first 10 spawns of ≥ 50 eggs in each aquarium were incubated and the percent hatch was determined. After hatching of the F1 embryos 2 x 25 newly hatched larvae groups were established in each aquarium as the spawning activity permitted. After 30 days post hatch exposure of F1 each larval group was terminated. The growth (individual length and wet weight) were measured and percent survival for each group recorded. The exposure of F_0 fish was terminated after 278 days. Each fish was measured (wet weight and length) and examined to verify sex and gonadal conditions. Additionally deformities or injuries were noted.
During the study newly hatched larvae were fed live brine shrimp nauplii three times daily, juvenile and adult fish were fed twice daily: frozen brine shrimp and "Ziegler® Brother Prime" flakes.
The following water quality parameters were monitored: Temperature, dissolved oxygen and pH were measured daily, and total hardness and specific conductivity were measured weekly.
During the chronic study, samples for chemical analyses of fluazinam in test solutions the test solution in each aquarium on the upper level was sampled a minimum of once each week, until the spawning (lower) level of the system was activated. Subsequently, test solution samples were taken weekly (minimum) from one replicate aquaria of each treatment level from the corresponding upper and lower level.
Findings:
Mean measured exposure concentrations were 0.69, 1.4, 2.9, 6.4 and 14 µg/L, which averaged 61 % of nominal concentrations. All biological endpoints are based on mean measured concentrations. The results of water quality parameters were: 24 – 25 °C, 6.9 – 7.5 mg O_2/L, pH 6.7 – 7.6, 24 – 30 mg $CaCO_3$/L (total hardness) and a specific conductivity of 125 – 150 µmhos/cm.

Table 36: Survival, growth, and reproduction data after 278 days exposure to fluazinam

endpoints	Mean measured concentrations (µg/L)					
	control	0.69	1.4	2.9	6.4	14
F_0 generation						
Survival day 30 (%)	87	94	89	86	81	32*
Survival day 278 (%)[a)]	88	100	100	96	90	62*
Mean blotted wet weight (g) 61 d post hatch	0.588	0.569	0.584	0.664	0.608	NA

endpoints	Mean measured concentrations (µg/L)					
	control	**0.69**	**1.4**	**2.9**	**6.4**	**14**
Mean standard length (mm) 61 d post hatch	41	40	40	41	40	41[b)]
Mean standard length male (g) day 278	86	84	87	85	83	81[b)]
Mean standard length female (mm) day 278	69	67	66	66	65	67[b)]
Mean blotted wet weight male (g) day 278	8.4	7.8	8.3	7.6	7.2*	6.9[b)]
Mean blotted wet weight female (g) day 278	3.7	3.2	3.2	3.1	3.1	3.5[b)]
Eggs /mature female (nº)	760	1056	475	539	84	422
Eggs / spawning (nº)	89	98	80	83	35*	75*
Hatching success (%)	88	85	80[c)]	85	83	63*
F_1 generation						
Survival %	94	89	76	95	92	80
Hatching success (%)	88[d)]	89	78**	76**	93[e)]	24**
Mean standard length (mm)	30	30	30	30	29[e)]	26[e)]
Mean blotted wet weight (g)	0.25	0.26	0.26	0.25	0.23[e)]	0.17[e)]

* significantly different when compared to pooled control

** significantly different when compared to solvent control.

NA not applicable due to reduced survival

[a)] Calculation is based on 25 fish per replicate, which continued in exposure after 61 day post-hatch exposure

[b)] Values not statistically analysed due to significantly reduced survival

[c)] Significant reduction is not considered to be toxicant related, as the test concentrations 2x and 4x higher did not produce an adverse effect

[d)] Results only from solvent control

[e)] Only results of replicate "A" were analysed

Additional information to the statistical analysis of hatching success of F1:

The statistical analysis of the F1 hatching success data was performed with a standard (chi-square) contingency table test. However the authors of the study have indicated that this analysis is not appropriate for the experimental design used in this study and the high variations in the raw-data between replicates for the mentioned endpoint. Therefore a revised statistical analysis was presented which intended to account for the complexity of the test design and the specific data. The revised statistical analysis of the hatching success of the F1 generation resulted in a NOEC of 2.9 µg/L and a NOEC of 6.4 µg/L.

Table 37: Fish Full-Life-Cycle study: Summary of all assessed endpoints

endpoints (time)	NOEC [µg/L]	LOEC [µg/L]
F0 generation		
embryo hatching success, larval survival and growth		
F_0 hatching success (5 d)	6.4	14
F_0 survival (30 day post hatch)	6.4	14
F_0 mean length (30 day post hatch)	2.9	6.4
F_0 mean weight (61 day post hatch)	no effects until 6.4 µg/L, the next higher treatment level (14 µg/L) could not be statistically analyzed due to significantly reduced survival	
F_0 mean length (61 day post hatch)	no effects until 6.4 µg/L, the next higher treatment level (14 µg/L)	

endpoints (time)	NOEC [µg/L]	LOEC [µg/L]
	could not be statistically analyzed due to significantly reduced survival	
survival and growth of adults:		
F_0 survival (test termination)	6.4	14
F_0 mean male total length (test termination)[1)]	2.9	6.4
F_0 mean male wet weight (test termination) [1)]	2.9	6.4
reproductive success		
F_0 number egg/spawn	6.4	14
F_0 number spawns/females	2.9	6.4
F_0 number eggs/females	6.4	14
F1 generation		
embryo hatching success, larval survival and growth of F_1		
F_1 hatching success (5 d)	6.4	14
F_1 survival (30 day post hatch)	6.4	14
F_1 mean length (30 day post hatch)	14	> 14
F_1 mean weight (30 day post hatch)	14	> 14

[1)] for females no effects on growth until 6.4 µg/L treatment level were observed, the next higher treatment level (14 µg/L) could not be statistically analyzed due to significantly reduced survival.

Conclusion: The most sensitive endpoints of F_0 were mean length of larvae(30 days post hatch), mean total length and wet weight of males (test termination) and number spawns/female with a NOEC of 2.9 µg/L. The most sensitive endpoints of F_1 were the hatching success and survival with a NOEC of 6.4 µg/L.
Comment (RMS): Study considered acceptable.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Acute toxicity to aquatic invertebrates (IIA 8.2.4)
Reference: Shults, S. K., Brock, A. W. & Laveglia, J. (1992): Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5108-91-0418-TX-002
Test guideline: OECD 202
GLP: yes
Test item: Fluazinam techn. (IKF-1216): purity 100 %, Lot# 1030/91
Material and methods:
The acute toxicity of fluazinam to the waterflea *Daphnia magna* was studied under flow through conditions over a 48 hours exposure period. Twenty daphnids (< 24 h old, 10 daphnids per replicate) were exposed to five nominal concentrations (39, 65, 110, 180 and 300 µg/L), a control and a solvent control. During the exposure period water quality parameters were measured: 20 – 21 °C, 78 – 93 % O_2 saturation, pH 8.1 – 8.3, 170 mg/L $CaCO_3$.
Chemical analysis of fluazinam was done at initiation (0 h) and termination (48 h) of the study.
Findings:
Mean measured concentrations were 34, 54, 94, 150 and 260 µg/L. After 48 hours at the lowest concentration the immobility of daphnids was 5 %, however at the next higher concentration level (54 µg/L) no effects were observed. The effects at the lowest concentration were not related to the presence of the toxicant, therefore the NOEC was estimated to be 54 µg/L. At the highest tested concentration the immobility was 65 % and a clear dose/response relationship could be noted. The

slope of the concentration-response curve was calculated to be 2.8 and the EC_{50} was determined to be 220 µg/L (95%CL: 190 – 300 µg/L) by the moving average method.
Conclusion: EC_{50} (48 h): 220 µg/L and NOEC: 54 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference: van den Bogaaert, M., Farrelly, E., J. & Hamer, M. (1991): Fluazinam: Chronic Toxicity to *Daphnia magna*. Report No: RJ0974B
Test guideline: OECD 202
GLP: Yes
Test item: Fluazinam techn., purity: 98.1 %, batch no: not stated
Material and methods:
The chronic effects of fluazinam on the survival, reproduction and growth of *Daphnia magna* were determined. 10 replicates of one daphnid (< 24 hours old) per test concentration were incubated under static renewal conditions for 21 days with daily feeding (*Chlorella vulgaris* suspension) and observation. Test solutions were renewed every 2 days and samples of the freshly prepared and used test solutions were analysed for fluazinam. The nominal exposure concentrations were 0.0125, 0.025, 0.5, 0.1and 0.2 mg/L, additionally a water and a solvent (methanol) control were prepared. Following water quality parameters were recorded: The temperature was in the range of 18.5 – 20.5 °C, the pH was between 7.4 and 8.5, the dissolved oxygen was in the range of 8.2 – 10.1 mg/L in fresh solutions and in old solutions the lowest measured value was 2.4 and the highest 10.6 mg/L. The water hardness was in the range of 167 – 176 mg/L $CaCO_3$.
Findings:
The mean measured concentrations in freshly prepared solutions were 0.014, 0.029, 0.056, 0.098 and 0.202 mg/L (98 – 117 % of nominal) and in old solutions 0.007, 0.013, 0.026, 0.054 and 0.112 mg/L (52 – 57 % of nominal). Endpoints are based on nominal concentrations.
Low dissolved oxygen concentrations were measured in old test solutions and could be explained with increased microbial activity in older solutions, however there were no observable effects on daphnids. On day 21 at the highest tested concentration (0.2 mg/L) 50 % of adult daphnids had died. For controls and concentrations up to 0.05 mg/L 10 % mortality was recorded. At 0.1 mg/L 20 % of adult daphnids were dead, thus the NOEC (mortality) was 0.05 mg/L and LOEC (mortality) was 0.1 mg/L. The number of live young per daphnid was significantly affected at 0.1 mg/L, therefore, the 21 d NOEC was determined to be 0.05 mg/L. No effects on growth (length) were observed at 0.0125 mg/L, whereas at the next higher concentration level (0.025 mg/L) the growth was significantly influenced. Thus the 21 d NOEC for growth was 0.0125 mg/L.
Conclusion: 21 d NOEC (growth): 12.5 µg/L and LOEC: 25 µg/L; 21 d NOEC (mortality and reproduction): 50 µg/L and LOEC: 100 µg/L based on nominal concentrations.
Comment (RMS): Study considered acceptable.

Reference: Shults, S. K., Brock, A. W. & Laveglia, J. (1993): Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Report No. 5109-91-0419-TX-002
Test guideline: FIFRA 72-4
GLP: Yes
Test item: Fluazinam techn., purity: 96.8 %, batch no: 1030/91

Material and methods:
The study was performed to assess the chronic effects of fluazinam on *Daphnia magna*. Replicates of 4 x 10 daphnids (< 24 hours old) per test concentration were incubated under flow-through conditions for 21 days. The nominal exposure concentrations were 9.4, 19, 38, 75 and 150 µg/L, additionally a water and a solvent (acetone) control were prepared. Observations on the survival, growth (mean total length and dry weight) and reproduction of adults as well as the number of immobilized young were recorded. The following water quality parameters were measured during the study: The temperature was in the range of 19 - 21°C, the pH was between 7.9 and 8.2, the dissolved oxygen was in the range of 7.2 – 8.4 mg/L, the water hardness was 170 mg/L as $CaCO_3$ and the specific conductivity was 500 µmhos/cm. Chemical analyses of fluazinam in exposure solutions were performed weekly.
Findings:
The mean measured concentrations of fluazinam in test solutions were 8.9, 16, 33, 68 and 140 µg/L. Biological endpoints are based on mean measured concentrations.
After 21 days survival of adults was significantly effected at the highest tested concentration (140 µg/L), thus NOEC (mortality) was 68 µg/L and LOEC was 140 µg/L. Since the survival was significantly influenced at 140 µg/L, reproduction and growth data for this treatment level was excluded from statistical analyses for treatment effects. At lowest concentration level no effects on reproduction and growth were observed, Therefore the NOEC was 68 µg/L as well.
Conclusion:
21 d NOEC (mortality, growth, reproduction): 68 µg/L and LOEC: 140 µg/L based on mean measured concentrations.
Comment (RMS): Study considered acceptable.

5.4.3 Algae and aquatic plants

Reference: Smyth, D. V. & Tapp, J. F. (1987): PP192 (B1216): Determination of Toxicity to the Green Alga *Selenastrum capricornutum*. Report No. BL/B/3056
Test guideline: OECD 201
GLP: Yes
Test item: Fluazinam techn. (PP162): purity 97 %, batch no: not stated
Material and methods:
A test on growth inhibition of *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was performed with fluazinam under static conditions.
The algal cultures (1.0×10^4 cells/ml in culture media) were exposed to seven nominal concentrations: 0.01, 0.018, 0.032, 0.056, 0.1, 0.18 and 0.32 mg/L as well as to a dilution and a solvent control (acetone). The test samples were incubated for up to 96 hours under static conditions, at temperatures from 23.8 – 24 °C, pH 6.9 – 7.4, and continuous illumination (light intensity: 7200 Lux). Cell densities were determined after 24, 48, 76 and 96 hours by electronic particle counting using a Coulter Counter Model ZB. The calculation of test substance inhibiting the growth (biomass and growth rate) was done separately for each treatment in comparison to control. Chemical analyses of fluazinam were conducted for all treatment levels at the start and end of the testing.
Findings:
Mean measured concentrations were: 0.008, 0.015, 0.026, 0.048, 0.082, 0.15 and 0.2 mg/L. All biological endpoints are based on mean measured concentrations.
No significant inhibition of biomass and growth rate were observed in concentration up to 0.048 mg/L, therefore the NOEC was 0.048 mg/L for both endpoints. The 96 h E_bC_{50} was calculated to be 0.16 mg/L (95% CL 0.12 – 0.22 mg/L). The growth rate at each concentration was

relatively constant and at the highest tested concentration the inhibition was 13 %, therefore the E_rC_{50} was estimated to be > 0.22 mg/L.
Conclusion: 96 hour E_bC_{50}: 160 µg/L, E_rC_{50}: > 220 µg/L, NOEC: 48 µg/L based on mean measured concentrations
Comment (RMS): Study considered acceptable.

Effects on aquatic plants (IIA 8.2.8)
Reference: Boeri, R. & T.J. Ward (2001): IKF-1216: Toxicity to the Duckweed, *Lemna gibba.* Report No. 2129-SK
Test guideline: ASTM 1991, EPA OPPTS 850.4400
GLP: yes
Test item: Fluazinam techn., purity: 98.4 %, batch no: A626/1995
Material and methods:
The toxicity of fluazinam to the duckweed *Lemna gibba* was assessed in a static renewal system (solution renewals on day 3 and 5) over a 7 days exposure period. Three replicates of aquatic plants (12 fronds per replicate) in 20X-AAP media were exposed to seven nominal concentrations: 1.0, 2.0, 5.0, 10, 20, 40 and 80 µg/L as well as to a dilution control and a solvent control (DMF). Environmental conditions throughout the study were monitored: 23.8 – 25.7 °C, pH 7.5 – 7.6 (day 0), pH 9.3 – 10.2 (day 7) and continuous illumination with an intensity of 5030 – 5480 lux.
Total number of fronds and abnormal appearance of fronds was observed on day 0, 3, 5 and 7. Inhibition of frond growth (biomass and growth rate) was calculated by standard statistical methods relative to pooled control data. Chemical analyses of fluazinam were conducted on day 0, 3 and 5 of each freshly prepared test solution and old samples were analysed on day 3, 5 and 7.
Findings:
Mean measured concentrations of fresh solutions were 0.859, 1.73, 4.58, 7.96, 17.5, 35.9 and 69.1 µg/L test item corresponding to 80 to 92 % of the nominal concentrations. In old solutions fluazinam was found in amounts of 0.645, 1.25, 3.03, 5.82, 11.4, 21.3 and 37.5 µg/L corresponding to 46.9 – 64.5 % of nominal concentrations. Thus all biological endpoints were related to mean initial measured concentrations.
On day 7 no significant inhibition of frond growth (biomass: AUC) and fronds growth rate was observed at concentrations up to 35.9 µg/L compared to the pooled control data. At 69.1 µg/L the inhibition of growth rate was 14 % and the inhibition of biomass was 26 %, both values were significantly different when compared to control. Therefore the NOEC was 35.9 µg/L and LOEC was 69.1 µg/L. The E_bC_{50} and E_rC_{50} could not be calculated because inhibition of biomass and growth rate were < 50 % in all tested concentrations, thus the E_bC_{50} and E_rC_{50} were estimated to be > 69.1 µg/L, based on initial measured concentration.
Conclusion: 7 d E_bC_{50} and E_rC_{50}> 69.1 µg/L, NOEC = 35.9 µg/L based on initial measured concentrations
Comment (RMS): In order to obtain a clear concentration response curve and a reliable EC50 the inhibition at highest tested concentration should be at least 50 %. Thus the study is considered not acceptable. However, fluazinam is a fungicide and a study with a higher plant species is not necessary according to the directive 91/414/EEC. Therefore the results will be accepted as additional information and there is no need to perform a new study.

5.4.4 Other aquatic organisms (including sediment)

Chronic toxicity to sediment dwelling organisms (IIA 8.2.7)
Reference: Stewart, K.M. & Shillabeer, N. (1997): Fluazinam: Determination of the Effects on Emergence of *Chironomus riparius*. Report No. BL6115/B

Test guideline: Proposed BBA Guideline 1995
GLP: yes
Test item: Fluazinam techn., purity: 97.9 %, batch no: AD0408
Material and methods:
The toxicity of fluazinam to sediment dwelling larvae *Chironomus riparius* was investigated in a 28 day static sediment toxicity test. For each tested treatment (3.13, 6.25, 12.5, 25, 50 and 100 µg/l), 3 biological replicates and 1 sample for chemical analyses were prepared containing 245 g of an artificial sediment (2 cm depth) and 1700 mL overlying water (15.5 cm water layer). After a standing period of 7 days 25 first instar larvae (2 days post hatch) were applied to each test vessel. One day after the addition of the test organisms the test substance was applied in required quantities to the overlaying water and test media were carefully mixed without disturbing the sediment. Observations were made daily for emergent adults and at test termination replicates without 100 % emergence were examined for number of live and dead larvae and pupae. During the test the following water quality parameter were reported: The temperature ranged from 19.4 to 20.1°C, the pH values were in the range of 7.6 – 8.1, the dissolved oxygen concentration ranged from 7.8 – 9.4 mg O_2/L, the water hardness was in the range of 82 – 102 mg $CaCO_3$/L and the conductivity increased from 368 µS/cm (day 0) to 482 µS/cm (day 28).
Findings:
Chemical Analysis: On day 0 mean measured concentrations of fluazinam in overlaying water were 3.27, 6.05, 13.1, 23.7, 44.9 and 88.2 µg/L (88 – 105 % of nominal concentrations). After 7 days exposure the mean measured concentrations ranged from 3 – 4 % of nominal (in three highest treatments) and at test termination the fluazinam concentrations were below the limit of detection. Thus all biological endpoints are based on nominal concentrations applied to overlaying water.
Biological data: Data for males and females were pooled for all evaluations, because no significant differences were found in the sex distribution of adults after 28 days. The time to first emergence and time to 50 % emergence were significantly influenced at the to highest concentration levels (50 and 100 µg/L). The total emergence after 28 days was not reduced at concentrations up to 6.25 µg/L. Thus the NOEC and LOEC for emergence are 6.25 µg/L and 12.5 µg/L. The 28 d EC50 (total emergence) was determined to be 77 (69 – 86) µg/L.
Conclusion:
28 d NOEC (emergence): 6.25 µg/L and LOEC: 12.5 µg/L, 28 d EC_{50} (emergence): 77 (69 – 86) µg/L, based on nominal concentrations
Comment (RMS): Study considered acceptable.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008, fluazinam has to be classified as:

R50 and H400

The lowest acute toxicity value was LC_{50} = 0.036 mg/L determined with *Oncorhynchus mykiss* (Gelin & Laveglia 1992). LC_{50} was ≤0.1 mg/L therefore Fluazinam fulfills the criteria for the proposed classification as R50 according to Directive 67/548/EEC and the criteria for the proposed classification as H400 according to Regulation EC 1272/2008. A M-factor of 10 is applicable based on 0.01 <L(E)C50 ≤0.1 mg/l.

R53

The classification is based on the fact that the active substance is not ready biodegradable (Grützner, 2000) and fulfills the criteria for the proposed classification as R53 according to Directive 67/548/EEC.

H410

follows from the rapid degradability in a water/sediment study with a $DT_{50\ whole\ sys.}$:
<16 d ($DT_{50\ whole\ sys.}$: phenyllab.: 4.3 d; pyridyllab.: 4.6 d) and there are adequate chronic toxicity data available for all three trophic levels. The lowest chronic toxicity value was the $NOEC_{F0\ growth,\ F1\ survival}$ = 0.0029 mg/L determined with *Pimephales promelas* (Shults et al. 1995). As the NOEC-value was ≤0.01 mg/L Fluazinam fulfills the criteria for the proposed classification as H410 according to Regulation EC 1272/2008.

	Classification and labelling according to	Criteria according to	Classification and labelling according to	Criteria according to
	Directive 67/548/EEC		Regulation EC 1272/2008.	
Acute (short-term) aquatic hazard	R50	LC_{50} ≤0.1 mg/L	H400	LC_{50} ≤0.1 mg/L
Long-term aquatic hazard	R53	active substance is not ready biodegradable	H410	Rapid degradation; adequate chronic toxicity data available for all three trophic levels; lowest chronic toxicity NOEC-value was ≤0.01 mg/L

Conclusion of environmental classification according to Directive 67/548/EEC

N	Follows from R 50/53
R50	Follows from the toxicity to fish (*Oncorhynchus mykiss* LC_{50} = 0.036 mg/L, Gelin & Laveglia 1992).
R53	Is based on the fact that the active substance is not ready biodegradable (Grützner, 2000).

Fluazinam therefore fulfills the criteria for classification following Directive 67/548/EEC. Fluazinam should be classified Dangerous for the Environment with the following risk and safety phrases:

N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment

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.

Conclusion of environmental classification according to Regulation EC 1272/2008

Regarding environment, H400 (very toxic to aquatic organisms) and H410 (Very toxic to aquatic life with long lasting effects**)** classification is proposed.

H400 follows from the acute toxicity to fish (*Oncorhynchus mykiss* LC_{50}= 0.036 mg/L, Gelin & Laveglia 1992),

H410 is based on the rapid degradability in a water/sediment study with a DT_{50} whole sys.: < 16 d (DT_{50} whole sys.: phenyllabelled: 4.3 d; pyridyllabelled: 4.6 d) and on the chronic toxicity to fish (*Pimephales promelas* (Shults et al. 1995)) $NOEC_{F0\ growth,\ F1\ survival}$= 0.0029 mg/L

Fluazinam therefore fulfills the criteria for classification as aquatic environmental hazard based on the CLP Regulation.

Fluazinam should be classified:

Aquatic Acute 1	**H400**	**‘Very toxic to aquatic life’**
Aquatic Chronic 1	**H410**	**‘Very toxic to aquatic life with long lasting effects’**

Signal Word: ‘Warning’ and environmental warning label.

A M-factor of 10 is applicable based on 0.01 <L(E)C_{50} ≤0.1 mg/l

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Fluazinam may be considered hydrolytic stable under acidic conditions. Under neutral and alkaline conditions it is rapidly hydrolysed.

^{14}C-phenyl labelled and ^{14}C-pyridyl labelled fluazinam rapidly degraded during aqueous photolysis at pH 5 (sterile buffer solution) at 25° C. The half life was calculated to be 2.5 days for both labels.

Fluazinam is of high toxicity to aquatic invertebrates, algae and fish.

The lowest acute toxicity value was the **LC_{50} = 0.036 mg/L** determined with *Oncorhynchus mykiss* (Gelin & Laveglia 1992).

Fluazinam is not readily biodegradable in a 28-day Manometric Respirometry test but shows a **rapid degradation** in a water/sediment study, with a $DT_{50\ whole\ system}$ with 4.3/4.6 days.

The lowest chronic toxicity value was the **$NOEC_{F0\ growth,\ F1\ survival}$ = 0.0029 mg/L** determined with *Pimephales promelas* (Shults et al. 1995).

Classification categories	aquatic environmental hazard **acute category 1** aquatic environmental hazard **chronic category 1**	
GHS Pictogram		
Signal Word	Warning	
Hazard Statement	H400	'Very toxic to aquatic life',
	H410	'Very toxic to aquatic life with long lasting effects'
	EUH401	'To avoid risks to human health and the environment, comply with the instructions for use'
M-factor	10	
Precautionary statements — Prevention	P273	Avoid release to the environment
	P391	Collect spillage
	P501	Dispose of contents/container to ….

M factor of 10 is applicable based on 0.01 <L(E)C_{50} ≤0.1 mg/l

RAC evaluation of environmental (aquatic) hazards

Summary of the Dossier Submitter's proposal

The dossier submitter proposed Aquatic Acute 1 (H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) (no M-factor proposed) according to CLP. The proposed classification according to Directive 67/548/EEC was N; R50/53 without specific concentration limits.

Degradation and bioaccumulation

Hydrolysis

Fluazinam is hydrolytically stable in acidic conditions, while under neutral conditions it is rapidly hydrolysed with DT_{50} values in the range 2.7 – 4.5 d. Its major metabolite CAPA is steadily hydrolyzed to DCPA with a DT_{50} value of about 32 days. DCPA is resistant to further degradation.

Photolysis

Fluazinam undergoes rapid aquatic photolytic degradation with DT_{50} = 2.5 d. The multitude of photolytic degradation products result from a complex degradation pathway with reduction and hydrolysis of NO_2, Cl and CF_3 substituents, the cleavage between the ring systems, ring opening and oxidative fragmentation with CO_2 production. The only major metabolite is G-504 (max. 17.1 % after 10 days). CO_2 production was 17.7 % after 30 days of exposure to simulated sunlight, indicating low ultimate photodegradation.

Biotic degradation

The substance is not readily degradable under test conditions. In a "28-Day-Manometric Respirometry Test" after 28 days the BOD in the test flasks was 12 and 14 mg O_2/l (arithmetic mean 13 mg O_2/l). The biodegradation rate was 1 %, based on $ThOD_{NH4,}$ and 0 %, based on $ThOD_{NO3}$.

In water-sediment study fluazinam was rapidly degraded with a DT_{50} in the whole system in the range from 3.1 to 5.7 d. The metabolite AMPA was reported as the major metabolite in sediment and was degraded with DT_{50} value of 33.9 days ("Emperor" sediment).The mineralization to CO_2 was low with maximal amounts of 2.2 % at day 100 indicating very low ultimate degradation.

Aquatic bioaccumulation

At 25ºC, the log K_{ow} of fluazinam is 4.19 (pH 4 to 7), indicating a potential for bioaccumulation

In addition, bioaccumulation test showed moderate bioaccumulation in fish, with a BCF of 960 - 1090 (whole fish). BCF was determined only for viscera and fillet, but was not corrected by lipid content.

Acute (short-term) aquatic toxicity

The results of short-term aquatic toxicity data for fish, crustaceae and algae are summarized in the table below. According to these studies, fluazinam is of high toxicity for all taxonomic groups, with a lowest EC_{50} value of 0.036 mg/l for fish (*Oncorhynchus mykiss*), based on measured concentrations. This short-term aquatic toxicity study was conducted at pH 6.8 to 7.1. This implies that the un-dissociated form was dominant (pKa=7.34), and therefore it is likely to be conservative, because fluazinam generally showed lower toxicity at basic pH.

Data element: Acute (short-term) aquatic toxicity of the active substance Fluazinam Generally expressed in terms of LC_{50} or EC_{50} (mg/l)				
	$L(E)C_{50}$ [mg/l]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC_{50}):				
*Oncorhynchus mykiss***	**0.036***	FIFRA Guideline 72-1	y	y
Crustacea (48 hr EC_{50}):				
*Daphnia magna***	0.220*	OECD 202	y	y
Algae (72 or 96 hr E_rC_{50}):				
*Pseudokirchn. Subcapitata****	> 0.220*	OECD 201	y	y
Conclusion: relevant endpoint for classification is LC/EC_{50} = 0.036 mg/l (measured pH 6.8 – 7.1)				

* Based on the mean measured concentrations

**Toxicity tests on fish and Daphnia were conducted under flow-through conditions with verification of fluazinan concentration.

***Algae test were conducted under static conditions with verification of fluazinan concentration.

Chronic (long-term) aquatic toxicity

The results of long-term aquatic toxicity data for fish, crustaceae and algae, summarized in the table below, show that fluazinam is highly toxic for all taxonomic groups. The relevant endpoint for chronic classification is the NOEC for fish (*Pimephales promelas)* . This value is $NOEC_{F0\ growth}$ = 0.0029 mg/l, based on mean measured concentration. This long-term toxicity study, as well as the short term study, was conducted at pH 6.8 to 7.1 and is considered to be conservative, because fluazinam generally showed lower toxicity at basic pHs.

Data element: Chronic (long-term) aquatic toxicity of the active substance Fluazinam

Generally expressed in terms of NOEC (mg/l)

	NOEC [mg/l]	Test guideline / design	GLP (y/n)	Reliability
Fish (34 d $NOEC_{F0\ growth}$)				
*Pimephales promelas***	**0.0029***	FIFRA Guideline 72-5	y	y
Crustacea (21 d $NOEC_{growth,}$):				
*Daphnia magna***	0.0125	OECD 202 (1984)	y	y
Algae (96 h NOEC):				
*Pseudokirchneriella subcapitata****	0.048*	OECD 201	y	y

Conclusion: relevant endpoint for classification is $NOEC_{F0\ growth,\ F1\ survival}$ = 0.0029 mg/l (measured pH 6.7–7.6)

* Based on the mean measured concentrations

**Toxicity tests on fish and Daphnia were conducted under flow-through conditions with verification of fluazinam concentration.

***Algae test were conducted under static conditions with verification of fluazinan concentration.

Aquatic toxicity of degradation products

Acute toxicity data are available for AMPA, the major metabolite resulting from biodegradation. This substance is poorly soluble and no acute toxicity is recorded at levels up to the water solubility. AMPA is not rapidly degradable (DT_{50} = 33.9 d ("Emperor" sediment)) and no experimentally determined BCF or log K_{ow} values are available. There are no data on chronic toxicity for this substance.

No data on aquatic toxicity of DCPA (metabolite formed in hydrolysis) and G-504 (metabolite formed in photolysis) are available.

Comments received during public consultation

Several comments were received during public consultation concerning the degradation of fluazinam. The comments proposed the consideration of fluazinam as non-rapidly degradable due to low mineralisation, and the setting of a chronic M-factor of 10.

The dossier submitter agreed with the comments and amended the CLH report (version 3) considering fluazinam as non–rapidly degradable according to CLP criteria and keeping the M-factor of 10 for chronic toxicity. In addition, information on the aquatic toxicity of AMPA was added in the CLH report.

Also it was recommended to correct the BCF for the whole fish in relation to the lipid content of test fish, but this information is not available in DAR.

RAC assessment - comparison with the classification criteria and justification

For presentation purposes the following table is placed outside the RAC box:

Endpoint	Classification Criteria		Evidence for Fluazinam
	CLP (2nd ATP)	**DSD**	
Degradation Fluazinam	Fluazinam it is rapidly hydrolysed with DT_{50} values in the range 2.7 – 4.5 under environmental relevant conditions. DCPA the stable main metabolite was found in amounts of 70.9 % (label I, day 56) and 38 % (label II day 28) of the applied radioactivity. Fluazinam is not readily biodegradable under OECD 301F test conditions within 28 days (pH 7.4). In water/sediment studies Fluazinam was degraded with a DT_{50} in the whole system in the range from 3.1 to 5.7 d. The metabolite AMPA was reported as major metabolite with amounts of max. 26.7 % AR (maximum of phenyl label; system 1, day 14) in sediment and was degradated with DT_{50} value of 33.9 days (average both labels; 24 days (phenyl label) and 43.7 days (pyridyl label); "Emperor" sediment. Although AMPA is the major metabolite, other metabolites are formed for which no data has been provided and thus it has not been demonstrated that they do not meet the criteria for classification. The mineralization to CO_2 was low with maximal amounts of 2.2 % at day 100 indicating very low ultimate degradation.		Fluazinam is **not readily biodegradable** under OECD 301F test conditions within 28 days. Fluazinam indicates primary degradation in abiotic degradation tests and in the water/sediment study, but ultimate degradation is low in any of these degradation studies. Due to -the low ultimate degradation of Fluazinam -missing data on aquatic toxicity of DCPA (metabolite formed in hydrolysis) as well as other metabolites formed in the water – sediment study. - **Fluazinam is not rapidly degradable.**
Bioaccumulation Fluazinam	**BCF > 500** (960 – 1090) **log K_{ow} is > 4** (4.19 at	**BCF > 100** (960 – 1090) **log K_{ow} is > 3**	The BCF* and the log K_{ow} exceeds the classification criteria for Directive 67/548/EEC as well as for

Endpoint	Classification Criteria		Evidence for Fluazinam
	CLP (2nd ATP)	**DSD**	
	pH 4 - 7)	(4.19 at pH 4 - 7)	CLP indicating a **potential for bioaccumulation.** *In the DAR the BCF was determined only for viscera and fillet, but was not corrected by lipid content. The classification as **R53** according to Directive 67/548/EEC is based on the **non rapid degradation** and on the observed potential for bioaccumulation.
Acute aquatic toxicity Fluazinam	**$LC/EC_{50} \leq 1$ mg/l**		Fluazinam is of high acute toxicity to fish *(Oncorhynchus mykis*s) with a LC_{50} = 0.036 mg/l and fulfills the criteria for the proposed classification as **R50** according to Directive 67/548/EEC and the criteria for the proposed classification as **Aquatic Acute 1** (H400) according to Regulation EC 1272/2008. An **M-factor of 10** is applicable based on 0.01 <L(E)C_{50} ≤0.1 mg/l.
	Active substance Fluazinam		
	*Oncorhynchus mykis*s **LC_{50} = 0.036 mg/**l		
	Daphnia magna EC_{50} = 0.220 mg/l		
	Pseudokirchn. Subcapitata E_rC_{50} = 0.220 mg/l		
Chronic aquatic toxicity Fluazinam	**For non rapidly degradable substances:** **0.001 <NOEC ≤0.01 mg/l**		Fluazinam is not rapidly degradable and of high chronic toxicity to fish (*Pimephales promelas*) with a NOEC $_{F0\ growth}$ = 0.0029 mg/l. Therefore Fluazinam fulfills the criteria for the proposed classification as **Aquatic Chronic 1** (H410) according to Regulation EC 1272/2008. An **M-factor of 10** is applicable based on 0.001 < NOEC ≤ 0.01 mg/l
	Pimephales promelas	**$NOEC_{F0\ growth}$= 0.0029mg/l**	
	Daphnia magna	NOEC $_{growth}$ = 0.0125 mg/l	
	Pseudokirchn. subcapitata	NOEC = > 0.048 mg/l	

Endpoint	**CLP (2nd ATP)**		**Evidence for AMPA**
	CLP (2nd ATP)	**DSD**	
Degradation of metabolite AMPA	No studies on photolysis, Hydrolysis and ready biodegradability are available. In a water/sediment study AMPA was reported as major metabolite with amounts of max. 26.7 % AR (maximum of phenyl label; system 1, day 14) in sediment and was degradated with DT_{50} value of 33.9 days (average both labels; 24 days (phenyl label) and 43.7 days (pyridyl label); "Emperor" sediment]		Based on DT_{50} of 33.9 d in a water/sediment system**, AMPA should be considered as not rapidly degradable.**
Bioaccumulation of Metabolite AMPA	**BCF > 500** **log K_{ow} > 4**	**BCF > 100** **log K_{ow} > 3**	No experimentally determined BCF or log Kow data available
Acute aquatic toxicity of metabolite AMPA	**L(E)C_{50}s are above the water solubility; Water solubility ≤ 1 mg/l** *Brachydanio rerio* *Daphnia magna* *Scenedesmus subspicatus*		"**No acute toxicity**" as L(E)C50s are above the water solubility. Due to the low solubility of the test substance the tests could not be performed with higher test concentrations. No mortality, no sublethal effects or immobility or toxic effects were observed in the test concentrations.
Chronic aquatic toxicity of metabolite AMPA	**L(E)C_{50}s are above the water solubility ; Water solubility = <1 mg/l** (no chronic aquatic toxicity studies with fish or daphnia are available) Brachydanio rerio *Daphnia magna* *Scenedesmus subspicatus*		AMPA was poorly soluble and no acute toxicity is recorded at levels up to the water solubility. AMPA is not rapidly degradable (DT_{50} water/sediment = 33.9 d) and no experimentally determined BCF or log K_{ow} values are available. AMPA (Classification is based on acute aquatic toxicity data, no chronic aquatic toxicity studies with fish or daphnia are available) fulfills the criteria for the proposed classification as **R53** according to Directive 67/548/EEC and the criteria for the proposed classification as **Aquatic Chronic 4** (H413) according to Regulation EC 1272/2008.

RAC assessment, continued:

Conclusion:

RAC concludes that an environmental classification for fluazinam as, Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410) according to the CLP Regulation, with an M-factor of 10 for both acute and chronic categories; and as N; R50/53 according to Directive 67/548/EEC, is justified.

6 OTHER INFORMATION

7 REFERENCES

7.1 Physico-chemical properties

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**
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
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
Asai, N.	1991	IKF-1216 (Pure Grade) - Determination of Physical State. Ishihara Sangyo Kaisha, Ltd., Report No. 1216-90-06303-1 GLP: no unpublished	N	ISK
Atkinson, R.	1993	Estimation of Hydroxyl Radical Reaction Rate Constants: Fluazinam. Ricerca Inc., Report No. RIC 1832 GLP: no unpublished	N	ISK
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	Y	ISK

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		
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 unpublished	Y	ISK
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
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
Gallacher, A.C.	1997	IKF-1216 - UV-VIS Absorption. Ricerca, Inc., Report No. 4039-97-0017-AS-001 GLP: yes unpublished	N	ISK
Kimura, T.	1991	IKF-1216 (Pure Grade) - Determination of Color. Ishihara Sangyo Kaisha, Ltd., Report No. 91 0509KT GLP: no unpublished	N	ISK
Kimura, T.	1991	IKF-1216 (Pure Grade) - Determination of Odor. Ishihara Sangyo Kaisha, Ltd., Report No. 91 0510KT GLP: no unpublished	N	ISK
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

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**
McFadden, J.J.	2000	Henry's Law Constant for Fluazinam. Ricerca, Inc., Report No. F-150-A GLP: no unpublished	N	ISK
Oguri, M.	1991	IKF-1216 (Pure Grade) - Determination of Color. Ishihara Sangyo Kaisha, Ltd., Report No. 1216-90-06302-1	N	ISK
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
Sanders, J.M.	1993	Fluazinam (IKF-1216) (ASC-66825) – Solubility. Ricerca, Inc., Report No. 4039-91-0384-AS-001 GLP: yes unpublished	N	ISK
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
van der Gaauw, A.	2003	14C-Fluazinam: Hydrolysis at Three Different pH Values. RCC Ltd, Report No. 846211 GLP: yes unpublished	Y	ISK
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
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
van Rijsbergen,	2002	Determination of the Density of IKF-1216	Y	ISK

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**
L.M.		PAI. Notox B.V., Report No. 341123 GLP: yes unpublished		
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
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
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
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
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
van Rijsbergen, L.M.	2002	Determination of the Flammability of IKF-1216 TGAI. Notox B.V., Report No. 341191 GLP: yes unpublished	Y	
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

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
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Wadley, A.M..	1992	Fluazinam: Quantum Yield Calculation. Zeneca Report No. Not Available GLP: no unpublished	N	ISK

7.2 Human health hazard assessment

Amyes S. J.	IIA, 5.3.2	1983	Four-week toxicity study in mice Life Science Research Ltd., Essex, England Report No.: 83/ISK036/067 GLP: LSR Quality Assurance Unit Unpublished	Y	ISK
Andre J. C.	IIA, 5.1.1	1994	Study to measure the pharmacokinetics of phenyl-^{14}C-IKF-1216 in the blood of rats Ricerca, Inc., Department of Toxicology and Animal Metabolism, Ohio Report No.: 5319-92-0262-AM-001 GLP: yes Unpublished	Y	ISK
Andre J. C.	IIA, 5.1.1	1994	Study to evaluate the distribution and excretion of (phenyl-^{14}C)-IKF-1216 (^{14}C (B)-IKF-1216) in rats Ricerca, Inc., Department of Toxicology and Animal Metabolism, Ohio Report No.: 5304-92-0185-AM-001 GLP: yes Unpublished	Y	ISK

Andre J. C.	IIA, 5.1.1	1994	Study to evaluate the distribution and excretion of (phenyl-^{14}C)-IKF-1216 in rats following repeated dosing Ricerca, Inc., Department of Toxicology and Animal Metabolism, Ohio Report No.: 5317-93-0021-AM-001 GLP: yes Unpublished	Y	ISK
Beck M.	IIA, 5.6.2	2006	B-1216: Teratology study in the rat Report No.: WIL-282006 GLP: yes Unpublished	Y	ISK
Broadmeadow A.	IIA, 5.3.1	1983	Four-week toxicity study in dietary administration to CD rats Life Science Research Ltd., Essex, England Report No.: 82/ISK035/544 GLP: LSR Quality Assurance Unit Unpublished	Y	ISK
Broadmeadow A.	IIA, 5.3.1	1985	B-1216: 13-week liver toxicity and 4-week reversibility study in dietary administration to CD rats Life Science Research Ltd., Suffolk, England Report No.: 84/ISK045/581 GLP: yes Unpublished	Y	ISK
Broadmeadow A.	IIA, 5.3.1	1985	B-1216: 13-week toxicity study in dietary administration to CD rats Life Science Research Ltd., Suffolk, England Report No.: 84/ISK046/635; Amended Final Report No.: 91/ISK046/0830; Addendum 3 GLP: yes Unpublished	Y	ISK
Broadmeadow A.	IIA, 5.3.3	1984	Four-week preliminary toxicity study in oral administration to beagle dogs Life Science Research Ltd., Suffolk, England Report No.: 84/ISK038/140; 85/ISK038/050 (Addendum I); 85/ISK038/248 (Addendum II) GLP: LSR Quality Assurance Unit Unpublished	Y	ISK

Broadmeadow A.	IIA, 5.3.3	1985	13-week toxicity study in oral administration to beagle dogs Life Science Research Ltd., Suffolk, England Report No.: 84/ISK048/692; Amended Final Report No.:91/ISK048/0832; Addendum 3 GLP: yes Unpublished	Y	ISK
Broadmeadow A.	IIA, 5.3.3	1987	52-week toxicity study in oral administration to beagle dogs Life Science Research Ltd., Suffolk, England Report No.: 86/ISK055/512; Addendum 1 GLP: yes Unpublished	Y	ISK
Bruynzeel D. et al	IIA, 5.9.2	1994	Contact dermatitis due to a new fungicide used in the tulip bulb industry Contact Dermatitis 1995: 33, 8-11	N	
Chambers P. R.	IIA, 5.3.2	1994	Toxicity to mice by dietary administration for 4 weeks Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England Report No.: ISK49/921049, Addendum 1 – 4; GLP: yes Unpublished	Y	ISK
Chambers P. R.	IIA, 5.3.2	1998	Toxicity to mice by dietary administration for 4 weeks Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK49/921049, Addendum 5 GLP: yes Unpublished	Y	ISK
Chambers P. R.	IIA, 5.5.1	1993	B-1216: Toxicity to rats by dietary administration for two years Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK/43/920649 GLP: yes Unpublished	Y	ISK
Chambers P. R.	IIA, 5.5.2	1998	Potential tumorigenic effects in prolonged dietary administration to mice Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK50/950671 GLP: yes Unpublished	Y	ISK

Cummins H. A.	IIA, 5.2.1	1988	Acute oral toxicity study in the mouse Life Science Research Ltd., Suffolk, England Report No.: 87/ISK106/860 GLP: yes Unpublished	Y	ISK
Cummins H. A.	IIA, 5.2.1	1988	Acute oral toxicity study in the rat Life Science Research Ltd., Suffolk, England Report No.: 87/ISK105/859 GLP: yes Unpublished	Y	ISK
Cummins H. A.	IIA, 5.2.2	1984	Acute percutaneous toxicity in the rat Life Science Research Ltd., Suffolk, England Report No.: 84/ISK051/586 GLP: yes Unpublished	Y	ISK
Cummins H. A.	IIA, 5.2.6	1984	Delayed contact hypersensitivity study in guinea-pigs Life Science Research Ltd., Suffolk, England Report No.: 84/ISK054/686 GLP: yes Unpublished	Y	ISK
Cummins H. A.	IIA, 5.3.4	1985	21-Day percutaneous toxicity study in CD rats Life Science Research Ltd., Suffolk, England Report No.: 84/ISK052/690; Amended Final Report No.:91/ISK052/0824 GLP: yes Unpublished	Y	ISK
Dawe S.	IIA, 5.3.2	1985	B-1216: Preliminary toxicity study in mice by dietary administration for 13 weeks Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK7/85172 GLP: yes Unpublished	Y	ISK
Hughes E. W.	IIA, 5.7	1997	IKF-1216: Neurotoxicity to rats by dietary administration for 13 weeks Huntingdon Life Sciences Ltd. Report No.: ISK 251/971800; GLP: yes Unpublished	Y	ISK

Hull R. M.	IIA, 5.3.3	1986	11-week oral toxicity study in dogs to investigate possible changes in retinal function and morphology and the reversibility of such changes Imperial Chemical Industries, PLC Report No.: CTL/C/1778 GLP: yes Unpublished	Y	ISK
Inouye T.	IIA, 5.8.1	1989	G-450: Micronucleus test in male mice The Institute of Environmental Toxicology Kodaira, Tokyo 187, Japan Report No.:IET 89-0015 GLP: yes Unpublished	Y	ISK
Inouye T.	IIA, 5.8.1	1989	G-450: Micronucleus test in female mice The Institute of Environmental Toxicology Kodaira, Tokyo 187, Japan Report No.:IET 89-0016 GLP: yes Unpublished	Y	ISK
Kajiwara Y.	IIA, 5.4.1	1988	Chromosomal aberration test of fluazinam technical using cultured mammalian cells Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1663E GLP: yes Unpublished	Y	ISK
Kitching J.	IIA, 5.4.1	2000	IKF-1216 Bacterial mutation assay Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England Report No.:RIA 015/003043 GLP: yes Unpublished	Y	ISK
Liggett M. P.	IIA, 5.2.1	1988	Acute oral toxicity to rats of B-1216 technical Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.: 881246D/ISK20/AC GLP: yes Unpublished	Y	ISK

Liggett M. P.	IIA, 5.8.1	1988	Acute oral toxicity to mice of G-450 Huntingdon Research Centre Ltd., Suffolk, England Report No.: 881245D/ISK19/AC GLP: yes Unpublished	Y	ISK
Liggett M. P.	IIA, 5.8.1	1988	Acute oral toxicity to mice of G-525 Huntingdon Research Centre Ltd., Suffolk, England Report No.: 881248D/ISK19/AC GLP: yes Unpublished	Y	ISK
Liggett M. P.	IIA, 5.8.2	1988	Acute oral toxicity to rats of G-624 Huntingdon Research Centre Ltd., Suffolk, England Report No.: 881247D/ISK20/AC GLP: yes Unpublished	Y	ISK
Liu Y.	IIA, 5.1.1	1993	Pilot study to evaluate the excretion of radiolabel following a single oral dose of ^{14}C-IKF-1216 to rats Ricerca, Inc., Department of Toxicology and Animal Metabolism, Ohio Report No.: 5204-92-0034-AM-001 GLP: yes Unpublished	Y	ISK
Maebashi H.	IIA, 5.8.3	1988	Effects on biological function of fluazinam technical MECT Co. Ltd. and Matsumoto Dental College Report No.: FR-2501 GLP: Yes Unpublished	Y	ISK
Marciniszyn J.	IIA, 5.1.1	1995	Study of the biliary excretion of radiolabel following oral administration (phenyl-^{14}C)-IKF-1216 to male Sprague-Dawley rats Ricerca, Inc., Department of Toxicology and Animal Metabolism, Ohio Report No.: 5318-92-0321-AM-001 GLP: yes Unpublished	Y	ISK

Matsumoto K.	IIA, 5.4.2	1999	IKF-1216 technical: Micronucleus test in mice The Institute of Environmental Toxicology Kodaira, Tokyo 187-0011, Japan Report No.:IET 98-0139 GLP: yes Unpublished	Y	ISK
May K.	IIA, 5.8.1	2002	HYPA: Bacterial reverse mutation test Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK 270/024536 GLP: yes Unpublished	Y	ISK
Mayfield R.	IIA, 5.5.1	1988	B-1216: Potential carcinogenicity and chronic toxicity study in dietary administration to rats for 104 weeks Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK8/87263, Report and Addendums 1 - 7 GLP: yes Unpublished	Y	ISK
Mayfield R.	IIA, 5.5.2	1988	B-1216: Potential carcinogenicity study in dietary administration to mice for 104 weeks Huntingdon Research Centre Ltd., Huntingdon, Cambridgeshire, England Report No.:ISK9/87264 GLP: yes Unpublished	Y	ISK
McClanahan R.	IIA, 5.1.1	1995	Study to identify the metabolites of IKF-1216 (fluazinam) in rats Ricerca, Inc., Department of Environmental and Metabolic Fate, Ohio Report No.: 5306-92-0191-AM-002 GLP: yes Unpublished	Y	ISK
Nakashima N.	IIA, 5.8.2	1998	B-1457 (Impurity 5): Comparative study on Susceptibility to Neurotoxicity in mice, rats and dogs The Institute of Environmental Toxicology Kodaira, Tokyo 187, Japan Report No.: IET 98-0020 GLP: yes Unpublished	Y	ISK

Nomura M.	IIA, 5.8.2	1998	Various Impurities in Fluazinam technical: Toxicological effect on brain of mice following a single oral administration Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1375/1411/1486 GLP: no Unpublished	Y	ISK
Nomura M.	IIA, 5.8.2	1998	Impurity 5, an Impurity in Fluazinam technical: Toxicological effect on brain and optic nerves of mice following a single oral administration at various stages of animal age Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1480 GLP: no Unpublished	Y	ISK
Nomura M.	IIA, 5.8.2	1998	Impurity 5, an Impurity in Fluazinam technical: Sensitivity comparison on brain of mice and rats following 14 day oral administrations Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1481 GLP: no Unpublished	Y	ISK
Nomura M.	IIA, 5.8.2	1998	Impurity 5, an Impurity in Fluazinam technical: Sensitivity comparison on brain of rats and mice in 3 and 10 weeks old following 14 day oral administrations Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1492 GLP: no Unpublished	Y	ISK
Nomura M.	IIA, 5.8.3	1998	Fluazinam technical: Toxicological effect on brain of rats and its reversibility by dietary administration for 14 days followed by a 25 day recovery period Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1323 GLP: no Unpublished	Y	ISK

Nomura M.	IIA, 5.8.3	1998	Fluazinam technical: Toxicological effect on brain of mice and its reversibility by dietary administration for 4 or 28 days followed by a 56 day recovery period Ishihara Sangyo Kaisha, Ltd., Osaka, Japan Report No.: AN-1333 GLP: no Unpublished	Y	ISK
Nomura M.	IIA, 5.8.3	1998	Fluazinam: Overview Document on CNS Toxicological Finding due to an Impurity 5 in Fluazinam technical Ishihara Sangyo Kaisha, Ltd., Osaka, Japan	Y	ISK
Ohtsuka M.	IIA, 5.4.1	1988	Bacterial reverse mutation test of fluazinam technical Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1674E GLP: yes Unpublished	Y	ISK
Ohtsuka M.	IIA, 5.4.1	1989	Bacterial reverse mutation test of fluazinam technical Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1673E GLP: yes Unpublished	Y	ISK
Ohtsuka M.	IIA, 5.4.1	1988	DNA repair test of fluazinam technical in bacillus subtilis Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1595E GLP: yes Unpublished	Y	ISK
Ohtsuka M.	IIA, 5.8.1	1989	Bacterial reverse mutation test of G-450 Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1676E GLP: yes Unpublished	Y	ISK

Ohtsuka M.	IIA, 5.8.1	1989	Bacterial reverse mutation test of G-525 Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1677E GLP: yes Unpublished	Y	ISK
Ohtsuka M.	IIA, 5.8.2	1989	Bacterial reverse mutation test of G-624 Hita Research Laboratories, Chemical Biotesting Center Chemicals Inspection and Testing Institute, Japan Report No.:T-1740E GLP: yes Unpublished	Y	ISK
Pritchard V.	IIA, 5.2.6	1986	Skin sensitisation to the guinea-pig of both the purified and technical material Imperial Chemical Industries, PLC, Cheshire, UK Report No.: CTL/P/1493 GLP: yes Unpublished	Y	ISK
Ransome S.	IIA, 5.4.1	2000	IKF-1216 Mammalian cell mutation assay Huntingdon Life Sciences Ltd., Huntingdon, Cambridgeshire, England Report No.:RIA 017/004090 GLP: yes Unpublished	Y	ISK
Serrone D. M.	IIA, 5.7	1995	An acute neurotoxicity screening study in rats with technical fluazinam (IKF-1216) Ricerca, Inc. Department of Toxicology and Animal Metabolism Report No.: 5603-93-0075-TX-003 GLP: yes Unpublished	Y	ISK
Shults S. K.	IIA, 5.2.4	1992	Primary dermal irritation study in albino rabbits with IKF-1216 Ricerca, Inc., Ohio Report No.: 5016-91-0281-TX-001 GLP: yes Unpublished	Y	ISK
Shults S. K.	IIA, 5.2.5	1992	Primary eye irritation study in albino rabbits with IKF-1216 Ricerca, Inc., Ohio Report No.: 5016-91-0280-TX-002 GLP: yes Unpublished	Y	ISK

Tesh J. M.	IIA, 5.6.1	1987	B-1216: Effects upon reproductive performance of rats treated continuosly throughout two successive generations Life Science Research Ltd. Report No.: 87/ISK068/097 GLP: yes Unpublished	Y	ISK
Tesh J. M.	IIA, 5.6.2	1985	B-1216: Teratology study in the rabbit Life Science Research Ltd. Report No.: 85/ISK049/045 GLP: yes Unpublished	Y	ISK
Tesh J. M.	IIA, 5.6.2	1988	B-1216: Teratology study in the rabbit Life Science Research Ltd. Report No.: 86/ISK069/324 GLP: yes Unpublished	Y	ISK
Tobeta Y.	IIA, 5.2.3	1988	Acute inhalation toxicity test of fluazinam in rats Hita Research Laboratories, Japan Report No.: D-1775E GLP: yes Unpublished	Y	ISK
Tominaga K. et al	IIA, 5.9.1	1990	Systemic contact dermatitis due to fluazinam Skin Research 1991: 33 (suppl 11) 364-368	N	
Van Ginkel C. et al	IIA, 5.9.2	1994	Allergic contact dermatitis from the newly introduced fungicide fluazinam Contact Dermatitis 1995: 32, 160-162	N	
Willoughby C. R.	IIA, 5.6.2	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

7.3 Environmental hazard assessment

7.3.1 Fate and Behaviour in the environment

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Bharti H., Bewick, D.W.	1985	B-1216 (PP192): Degradation in Soil. ICI Plant Protection Division, Report No. RJ0444B. GLP, unpublished	N	ISK
Bharti H., Bewick, D.W.	1985	B-1216 (PP192): Degradation in Soil. ICI Plant Protection Division, Report No. RJ0444B. GLP, unpublished	N	ISK
Burke, S. R., Sapiets, A.	1992	Fluazinam: Soil Dissipation Study (Germany, 1991-1992). ICI Agrochemicals, Report No. RJ1368B GLP, unpublished	N	ISK
Burke, S. R., Sapiets, A.	1993	Fluazinam: Residue Levels of the Metabolite R270682 (“HYPA”) in Soil From a Dissipation Study Carried Out in Germany During 1991-1992. ICI Agrochemicals., Report No. RJ1443B GLP, unpublished	N	ISK
Burke, S. R., Sapiets, A.	1992	Fluazinam: Soil Dissipation Study (Germany, 1991-1992). ICI Agrochemicals, Report No. RJ1368B GLP, unpublished	N	ISK
Burke, S. R., Sapiets, A.	1993	Fluazinam: Residue Levels of the Metabolite R270682 (“HYPA”) in Soil From a Dissipation Study Carried Out in Germany During 1991-1992. ICI Agrochemicals., Report No. RJ1443B GLP, unpublished	N	ISK
Crawford, C. J., Dillon, K. A.	1995	Dissipation of Residues of Fluazinam and Its Metabolites (MAPA, HYPA and CAPA) from Soil in Washington. Ricerca, Inc., Report No. 5687-93-0091-CR-001 GLP, unpublished	N	ISK
Crawford, C. J., Dillon, K. A.	1995	Dissipation of Residues of Fluazinam and Its Metabolites (MAPA, HYPA and CAPA) from Soil in North Dakota. Ricerca, Inc., Report No. 5687-93-0111-CR-001 GLP, unpublished	N	ISK

Crawford, C. J., Dillon, K. A.	1995	Dissipation of Residues of Fluazinam and Its Metabolites (MAPA, HYPA and CAPA) from Soil in California. Ricerca, Inc., Report No. 5687-93-0108-CR-001 GLP, unpublished	N	ISK
Crawford, C. J., Dillon, K. A.	1995	Dissipation of Residues of Fluazinam and Its Metabolites (MAPA, HYPA and CAPA) from Soil in Georgia. Ricerca, Inc., Report No. 5687-93-0104-CR-001 GLP, unpublished	N	ISK
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7.3.2 Aquatic Toxicity

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Gelin, M.D, Laveglia, J.	1992	Technical Fluazinam (IKF-1216) – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions. Generated by: Springborn Laboratories, USA Report No: 5099-91-0422-TX-002 GLP / GEP: yes unpublished	N	ISK
Gelin, M.D, Laveglia, J.	1993	Technical Fluazinam (IKF-1216) – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. Generated by: Springborn Laboratories, USA Report No: 5099-91-0421-TX-002 GLP / GEP: yes unpublished	N	ISK
Hertl, A.	1997a	Acute Toxicity of AMPA to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Static Test. Generated by: RCC Umweltchemie AG, Switzerland, Report No: 662512 GLP / GEP: yes unpublished	N	ISK
Hertl, J.	1997b	Acute Toxicity of AMPA to *Daphnia magna* in a 48-Hour Immobilization Test. Generated by: RCC Umweltchemie AG Report No. 662490 GLP / GEP: yes unpublished	N	ISK

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Hill, R. W.	1985	PP192: Determination of Acute Toxicity to Rainbow Trout (*Salmo gairdneri*). Generated by: ICI Brixham Laboratory, UK Report No: BL/B/2560 GLP / GEP: yes unpublished	N	ISK
Lentz, N. R., Huhtanen, K. L.	1994	Uptake, Depuration, and Bioconcentration and Metabolism of (Fluazinam) Carbon-14 IKF-1216 in Bluegill Sunfish (*Lepomis macrochirus*) Under Flow Through Test Conditions. Generated by: ABC Laboratories Report No. 5311-93-0013-EF-001 GLP / GEP: yes unpublished	N	ISK
Peither, A.	2001a	Acute Toxicity of Fluazinam to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Flow-Through Test. Generated by: RCC Ltd, Switzerland Report No: 813431 GLP / GEP: yes unpublished	Y	ISK
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Shults, S. K, A. W. Brock & L. Laveglia	1993	Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: Springborn Laboratories, USA Report No: 5017-91-0415-TX-002 GLP / GEP: yes unpublished	N	ISK
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
Shults, S. K., Brock, A. W., Laveglia, J.	1992	Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: Springborn Laboratories Report No. 5108-91-0418-TX-002 GLP / GEP: yes unpublished	N	ISK
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Smyth, D. V., Tapp, J. F.	1987	PP192 (B1216): Determination of Toxicity to the Green Alga *Selenastrum capricornutum*. Generated by: Imperial Chemical Industries PLC Report No: BL/B/3056 GLP / GEP: yes unpublished	N	ISK
Stewart, K.M., Shillabeer, N.	1997	Fluazinam: Determination of the Effects on Emergence of *Chironomus riparius*. Generated by: Zeneca Limited Brixham Environmental Laboratory Report No. BL6115/B GLP / GEP: yes unpublished	N	ISK

van den Bogaaert, M., Farrelly, E., J., Hamer, M.	1991	Fluazinam: Chronic Toxicity to *Daphnia magna*. Generated by: ICI Plant Protection Division Report No. RJ0974B GLP / GEP: yes unpublished	N	ISK

8 ANNEXES

none