Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

Fluazinam

EC number: not assigned
CAS number: 79622-59-6

ECHA/RAC/CLH-O-0000002667-66-01/A2

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Adopted

15 June 2012

Annex 2.1: Comments and response to comments on CLH Proposal and Justification

[ECHA has compiled the comments received via internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensive as possible. Please note that some of the comments might occur under several headings when splitting the given information is not reasonable.]

Substance name: Fluazinam
EC number:
CAS number: 79622-59-6

General comments

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	France / Member State	The fact that there is no classification for the following physico-chemical properties must be justified: explosive properties, flammability and corrosive properties.	Will be considered in revised CLH report as part of the RCOM (chapter 2.2 – short summary of the scientific justification for CLH proposal), see Annex 2.2	Corrected in revised CLH report as part of the RCOM, see Annex 2.2
01/07/2011	Germany /Member State	Comment on behalf of the German CA: Please check for correct page numbering.	noted	Corrected in revised CLH report as part of the RCOM, see Annex 2.2, .but not in the table of contents.
29/06/2011	Netherlands / RIVM / National Authority	In table 3 and 4, a current classification is included for fluazinam. However, as is also indicated in table 2, fluazinam is not yet included in Annex VI.	noted	Not corrected.
09/06/2011	Switzerland / Makhteshim Agan Holding	Dear members of the Risk Assessment Committee, Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, owns relevant data with regards to classification of	Studies will be presented in revised CLH report	It has been introduced and assessed in the

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td>B.V. (on behalf of Makhteshim Chemical Works Ltd.) / Company-Manufacturer</td><td>Fluazinam (=MCW 465) which we would like to add to the discussion. The data was generated in European Contract Research Institutes in 2006/2009 under GLP.

The acute oral and dermal toxicity studies confirm that no classification for these endpoints is required. The acute inhalation toxicity study indicates that no classification for this endpoint is required, as no mortality was observed up to the limit concentration. Also, no classification for acute local irritation or skin sensitization is supported by the respective studies.

(See attachment: overview on table 1, six individual study reports)

The differences in toxicological properties of technical Fluazinam evaluated for Annex I inclusion and Makhteshim's Fluazinam technical are potentially a consequence of 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 , but absent from Makhteshim's technical material (see EFSA Scientific review 2008, 137). Thus, the classifications proposed in the CLH report for the endpoints discussed above are not considered specific to Fluazinam itself and thus not appropriate.

With best regards,

Dr. Christian Strupp

Corporate Toxicologist, Makhteshim Agan Group
christian.strupp@ma-europe.com

ECHA comment: The document Makhteshim Chemical Works, Summary of acute toxicity of Fluazinam technical, Table 1. (table 1.docx) is copied below:

Table 1

Summary of acute toxicity of Fluazinam technical (Makhteshim Chemical Works)

<table>
<tr><th>Type of study</th><th>Species</th><th>Result</th><th>Reference</th></tr>
<tr><td>Acute oral LD_{50}</td><td>Rat,
CD® (Crl: CD®)</td><td>LD_{50} > 2000 mg/kg bw</td><td>Chevalier, F (2006); 19774/06, Sponsor report no. R-20269</td></tr>
</table>
</td><td>as part of the RCOM, see Annex 2.2</td><td>revised CLH report as part of the RCOM, see Annex 2.2

More information on the toxicity and classification of the impurity 5 would have been needed in the CLH report to assess the potential impact properly.</td></tr>
</table>

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Acute dermal LD_{50}</td><td>Rat,
CD® (Crl: CD®)</td><td>LD_{50} > 2000 mg/kg bw</td><td>Chevalier, F. (2006); 19775/06, Sponsor report no. R-20270</td></tr>
<tr><td>Acute inhalation LC_{50} (4h)</td><td>Rat,
HsdRCCHan^{TM} : WIST</td><td>LC_{50} inhalation rat > mean achieved atmosphere concentration of 4.82 mg/L</td><td>Griffiths, D. R. (2009), 0306/0391, Sponsor report no. R-24975</td></tr>
<tr><td>Acute skin irritation</td><td>Rabbit,
Himalayan</td><td>non-irritating</td><td>Leuschner, J. (2006); 19777/06, Sponsor report no. R-20272</td></tr>
<tr><td>Acute eye irritation</td><td>Rabbit,
Himalayan</td><td>non-irritating</td><td>Leuschner J. (2006); 19778/06, Sponsor report no. R-20273</td></tr>
<tr><td>Skin sensitisation – Magnusson & Kligman test</td><td>Guinea pig,
Dunkin-Hartley</td><td>non-sensitising</td><td>Chevalier, F. (2006); 19779/06, Sponsor report no. R-20274</td></tr>
</table>
ECHA comment: View document attached Griffiths D R, 2009, MCW 465 tech: Acute Inhalation Toxicity (Nose Only) Study in the Rat, Project Number 0306/0391, Sponsor Number R – 24975. (R-24975.pdf)
ECHA comment: View document attached Leuschner P, 2006, Acute Dermal Toxicity Study of MCW 465 In Rats, Laboratory of Pharmacology and Toxicology, Sponsor No R-20270 (R-20270.pdf)
ECHA comment: View document attached Leuschner P, 2006, Acute Oral Toxicity Study of MCW 465 in Rats, Laboratory of Pharmacology and Toxicology, Sponsor No R-20269, Germany (R-20269.pdf)
ECHA comment: View document attached Leuschner P, 2006, Acute Dermal Irritation/Corrosion Test (Patch Test) of MCW 465 in Rabbits, Laboratory of Pharmacology and Toxicology, Sponsor No R-20272, Germany (R-20272.pdf)
ECHA comment: View document attached Leuschner P, 2006, Acute Eye Irritation/Corrosion Test of MCW 465 in Rabbits, Laboratory of Pharmacology and Toxicology, Sponsor No R-20273, Germany (R-20273.pdf)
ECHA comment: View document attached Leuschner P, 2006, Examination of MCW 465 in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test), Laboratory of Pharmacology and Toxicology, Sponsor No R-20274, Germany (R-20274.pdf)
</td><td></td><td></td></tr>
</table>

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	United Kingdom / UK Competent Authority / MSCA	We were disappointed that the report was not written in accordance with the CLH report guidance and the format provided on ECHA's website. We recognise that the report must already have passed an accordance check but would question whether it is truly fit for the purpose it is intended. In several sections there is unnecessary detail making it difficult to identify key effects relevant for classification, whereas in other sections insufficient detail has been provided. We recommend that the main sections are limited to a discussion of effects potentially relevant to classification, that the key effects identified are summarised in the summary sections and that the 'comparison with criteria' sections contain a clear application of the criteria to these key effects.	We note that the CLH report on Fluazinam does not fulfil the expectations of UK colleagues. We kindly ask the UK colleagues to address their concerns about the accordance check directly to ECHA/RAC in order to bring forward the discussion on the amount of information "sufficient" for C&L purposes and to avoid future disappointments.	We agree that in some sections there is unnecessary information. Extensive information about the tests performed, such as very detailed descriptions of material and methods, is not relevant for classification purposes and therefore should not have been included in CLH report.
		In addition, the extent of detail provided for each study and hazard class is not consistent. For example, the information provided in the reproductive toxicity sections is extensive, whereas the repeated dose toxicity and carcinogenicity sections only include brief overviews of the findings (including limited information on the neoplastic findings).	Noted; Information provided in the reproductive toxicity sections is extensive because classification and labelling is proposed. Repeated dose toxicity and carcinogenicity	Rapporteurs agree with the comment.

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			sections only include brief overviews of the findings because classification and labelling is not proposed. This is in line with the instructions of the CLH report format of ECHA also. But for completeness, more information will be given in revised CLH report as part of the RCOM, see Annex 2.2	
		Please be consistent when presenting information as to whether a study has been conducted according to guidelines or not. Extensive information on the materials and methods (e.g. where test animals have been supplied from and their weight range etc.) are not required for studies conducted according to OECD guidelines, please remove this information from the CLH report.	Noted.	Noted.
		Overall, the inconsistencies in the level of detail provided in the report made it unacceptably difficult and time consuming to review. We would find it problematic to comment routinely if many more dossiers were to be of such quality. We recommend that the Austrian CA consider re-drafting much of this report to ensure that the basis for the proposal can be understood and then discussed effectively.	No fundamental re-drafting of the CLH report on Fluazinam can be conducted at this stage in process. We are convinced that the CLH report on Fluazinam as it is ensures that the	Agree. CLH report could have been improved.

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			basis for the proposal can be understood and discussed effectively.	
		Section 1.3: A large number of S-phrases have been proposed, please reconsider these as a number are not appropriate. In addition, they may need to be to be reconsidered in light of our comments on the proposed classification and labelling.	Section 1.3: Please give information which S-phrases are not appropriate in your opinion.	Noted.
04/07/2011	Finland / Member state	The proposal is difficult to read because the page numbers in the table of contents do not match with the page numbers in the text- please revise. Throughout the text the names of the hazard categories according to the CLP regulation are written incorrectly, for instance in section 4.4.1.4 the expression "acute hazard category 2" for skin irritation; in section 4.4.2.4 the expression "acute hazard category 1" for eye damage etc. Please use correct expressions for the hazard classes/categories of CLP. In the proposal some precautionary statements have been proposed, however this is not according to the CLP regulation. Also, both signal words have been proposed. Please delete the proposed P-statements and the signal word "Warning". It would improve the clarity if classification and labeling parts of the sections 1.2 and 1.3 would be separated.	Page numbers and names of the hazard categories will be corrected in revised CLH report as part of the RCOM, see Annex 2.2, and the signal word "Warning" will be removed.	Page numbers are corrected in revised CLH report as part of the RCOM, see Annex 2.2, but not in table of contents.
01/07/2011	Sweden / Member State	The Swedish Chemicals agency (KemI) supports the suggested classifications Editorial remarks Index pages numbers do not correspond to pages in the report. Tables in the reproduction toxicity section are mostly not numbered. Table references (page 76 and 77) in the environmental section do not match general table numbers.	Index pages numbers and pages in the report will be corrected and tables in the reproduction toxicity section will be numbered in revised CLH report as part of the RCOM, see Annex 2.2	Page numbers are corrected in revised CLH report as part of the RCOM, see Annex 2.2 but not in table of contents.

Carcinogenicity

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	United Kingdom / UK Competent Authority / MSCA	P45. Section 4.10.1. In order to assess the carcinogenic potential of fluazinam, all relevant tumour incidences identified (both adenoma and carcinoma) must be provided for each study along with any corresponding historical control data.	For completeness, more information will be given in revised CLH report as part of the RCOM, see Annex 2.2	Ok.
01/07/2011	Germany / Member State	Comment on behalf of the German CA: Based on the given information, the proposal seems justified not to classify fluazinam for carcinogenicity. We would appreciate it, however, if the complete dose-response relationship for tumour incidences was presented, i. e. including tumour incidences also in controls and referring both to adenomas and carcinomas.	For completeness, more information will be given in revised CLH report as part of the RCOM, see Annex 2.2	Noted.
29/06/2011	Netherlands / RIVM / National Authority	It is not clear from the CLH dossier whether liver cell tumours are observed in one or both of the mice studies. For a proper discussion whether based on these tumours fluazinam needs to be classified for carcinogenicity or not, more details on the actual incidences of these tumours/sex/dose (including controls) and on mortality should be included in the CLH dossier.	For completeness, more information will be given in revised CLH report as part of the RCOM, see Annex 2.2	Revisions have been included in revised CLH report as part of the RCOM, see Annex 2.2.

Mutagenicity

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
01/07/2011	Germany / Member State	Comment on behalf of the German CA: We agree with the proposal for non-classification.	Noted	Noted. The rapporteurs agree.
29/06/2011	Netherlands / RIVM / National Authority	Although the information in the table indicates that fluazinam is not genotoxic, more extensive study summaries are needed for a justified conclusion, including the use of controls, compliance to guidelines (all studies), number of animals and signs of exposure of the bone marrow (in vivo study).	For completeness, more information will be given in	Revisions have been included in revised CLH report

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			revised CLH report as part of the RCOM, see Annex 2.2	as part of the RCOM, see Annex 2.2

Toxicity to reproduction

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
01/07/2011	Spain / Member State	p. 65 Summary and discussion of reproductive toxicity Summary and discussion of Developmental toxicity The Spanish CA supports the proposed classification of Fluazinam as Xn; Repr. Cat. 3 R63 (Possible risk of harm to the unborn child) according to Directive 67/548/EC and as Repr. 2 (H361: Suspected of damaging the unborn child) according to Regulation EC 1272/2008. This proposal is based on the following effects in two species: • An Increased incidence of fetal abnormalities at 12 mg/kg bw/d observed in a rabbit teratology study (Tesh J.M., et al; 1988). Placental abnormalities, kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones were outside historical control values and occurred in presence of only slight maternal toxicity (↓6% bw). • An increased incidence of gross morphological fetal abnormalities (palatal cleft or diaphragmatic hernia) observed at 250 mg/kg bw/d in a rat teratology study (Willoughby C.R. et al; 1984). These effects were observed in presence of maternal toxicity (↓15% bw) and were outside the range of the concurrent controls and the recorded background controls of the laboratory. • An increased incidence of renal papillae not developed and distended ureter(s) observed at doses ≥ 50 mg/kg bw/d in a rat teratology study (Beck M; 2006). The values exceeded the maximum mean value in the historical control data and occurred in the absence of maternal toxicity. In this same study, statistically significant post-implantation losses increase, reduced ossification of the skull and vertebral arches and unossified sternebrae were observed at 300 mg/kg bw/d in presence of maternal toxicity.	Noted	Noted.
01/07/2011	Sweden / Member State	Swedish Chemicals Agency (KemI) supports Classification Toxic for reproduction category 3 according to Dir. 67/548/EEC and Reproductive toxicity category 2, according to Reg. 1272/2008 is supported. As the 2 generation study with rats conception rate and fertility index were slightly reduced in the F1 high dose group (500 ppm). Gestation length was slightly increased for the high dose group and number of implantation sites and litter sizes 4 days post partum were slightly reduced for F1 high dose and marginally for the intermediate group (100 ppm). Development toxicity studies performed in rabbits and rats showed indications of implantation losses.	Noted	Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Observations were made in rabbit fetuses of effects on placenta, bones in the head, incomplete ossification of and fused sternebrae in the top dose (12 mg/kg bw/d) and fetal abnormalities were observed at the top dose (250/300 mg/kg bw/d) in rats.		
01/07/2011	Germany/ Member State	Comment on behalf of the German CA: We agree that classification for developmental toxicity is needed. However, based on the findings in the two rat studies, it should be reconsidered if classification for reproductive toxicity in category 3; R63 (DSD) and category 2 – H361d (CLP), respectively, is justified. Considering occurrence of palatal clefts and diaphragmatic hernia in rat fetuses at a dose level of 250 mg/kg bw (Willoughby et al. 1984), significant signs of fetal 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), classification for reproductive toxicity in category 2; R61 (DSD) and category 1B – H360D (CLP), respectively, might be more appropriate. On the other hand, we are aware that 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 fetuses need to be balanced against maternal toxicity. Taking these considerations into account, we would therefore appreciate it if there was a more detailed explanation on what leads to the conclusion that Fluazinam should be classified as reproductive toxicant in category 3; R63 (DSD) and category 2 – H361d (CLP), respectively, and not in category 2; R61 (DSD) and category 1B – H360D (CLP), respectively.	In the study by Willoughby C.R. et al; 1984, a dose level of 250 mg/kg bw/d 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. Fetal and placental weights were significantly reduced in the 250 mg/kg bw/d 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	This is discussed in the opinion. Rapporteurs agree with the explanation of the dossier .

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			increased incidence of palatal cleft or diaphragmatic hernia were recorded at the top dose and values were outside the range of the concurrent controls and the recorded background controls of the laboratory. In the study by Beck M; 2006, there was no indication of teratogenicity, but a dose level ≥ 50 mg/kg showed an increased incidence of renal papillae not developed and distended ureters (1,6% and 2,5% /litter). The values exceeded the maximum mean value in the historical control data (0.8 %) and occurred in the absence of	

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			maternal toxicity. In the presence of maternal toxicity (300 mg/kg bw/d), an increase of post-implantation losses, reduced ossification of the skull and vertebral arches and unossified sternebrae were observed (statistically significant). Considering all these aspects, classification "Toxic for reproduction category 3" according to Dir. 67/548/EEC and "Reproductive toxicity category 2", according to Reg. 1272/2008 was proposed.	
29/06/2011	Netherlands / RIVM / National Authority	1)In the carcinogenicity studies in rats, testes effects (weight and histopathological changes) were observed. It should be discussed whether these effects are adverse enough for classification for fertility. 2)Page 61, conclusion and page 66 (4.11.4): Maternal body weight effects at 50 mg/kg bw are only marginal and not statistically significant. In addition, also the effects on fetal and placental weight at 50 mg/kg bw are not statistically significant. It should be discussed whether these effects are toxicologically relevant 3) Page 65-67, 4.11.4 and 4.11.5: According to the CLP criteria (3.7.2.3.3), small changes in fetal body weight or ossification are not enough for classification. The toxicological relevance of the observed fetal effects should be	Please refer to the explanation given to the comments of Germany	See answer to Germany.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		discussed. In addition, doses that induce fetal effects in the studies (including the higher doses that induce more severe effects as cleft palate in one of the rat studies) should be compared with maternal toxicity at the same dose and especially with the relevance of the maternal toxicity for the fetal effects to conclude whether the criteria of DSD and CLP are indeed fulfilled. For example, in the second rat teratology study, net body weight of mid dose females is only 98% of controls (and not significantly different). This is not likely to cause secondary effects on development. According to the CLP criteria (3.7.2.4.3), classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity.		
04/07/2011	Finland / MSCA	The indications of teratogenicity from reproductive toxicity studies supports the proposed classification: Reproductive toxicity, category 2 according to Annex I of Regulation (EC) No. 1272/2008); and category 3 according to Annex VI of Council Directive 67/548/EEC.	Noted	Noted.
04/07/2011	United Kingdom / UK Competent Authority / MSCA	Developmental toxicity P53, section 4.11.2.1. This section contains an excessive amount of information making it difficult to identify key effects. Please remove any information not relevant to classification (e.g. NOAELs). This makes it difficult to cross reference the key effects that have been mentioned in section 4.11.4. P 66 section 4.11.5. When comparing the results to the classification criteria, it would aid the reader if a justification as to why the data did not support classification in one of the other categories was included (e.g. category 1 was not justified as there is no evidence of developmental toxicity in humans etc.). In the rat studies, significant reductions in maternal weight gain were observed at the high dose levels. As such, at this does level, many of the minor effects observed (decreased foetal weight, foetal immaturity, delayed ossification, and under developed renal papillae and distended uterus) are likely to be a secondary consequence of maternal toxicity and not relevant for classification. Of the malformations observed, the kinked tail in the rabbit and cleft palate in the rat are of the most concern. In the rat, the cleft palate was only observed at a low incidence in one litter and was not observed in a second study conducted to similar dose levels. Therefore, it is arguable this effect was a chance finding. Similarly, the increased incidence of kink tail was only slightly higher than the historical control incidence and therefore may also have occurred by chance. Finally, it is difficult to assess the significance of the post-implantation loss observed in the Beck rat study, given that no historical control data has been provided. However, concern is reduced as a toxicologically relevant increase was not observed in the Willoughby study, conducted in the same strain at similar dose levels. In addition, the post-implantation loss incidence observed in the Beck study, was similar to the top of the historical	Please refer to the explanation given to the comments of Germany	See answer to Germany.

<table>
<tr><th>Date</th><th>Country/ Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>control range provided in support of the Willoughby study.

Overall, there is limited evidence supporting classification for developmental toxicity.</td><td></td><td></td></tr>
<tr><td>04/07/2011</td><td>Belgium / ISK Biosciences Europe N.V. / Company-Manufacturre</td><td>p. 66 comparison with criteria and p.67 conclusions on classification and labelling; company does not agree with Xn, R63 classification. Detailed reasoning why fluazinam should not be classified R63 is explained in attached documents.

ECHA comment: The document Letter from ISK Biosciences Europe N.V 04/07/2011, "CLH report – Proposal for classification and labeling of the active substance fluazinam", (IBE comment fluazinam classification proposal.pdf) is copied below:

Dear Sir, Madam,
Subject: CLH report – Proposal for classification and labelling of the active substance fluazinam
This document is aimed to address point 2.4.1 self-classification and labelling proposal based on the CLP Regulation criteria by the notifier ISK Biosciences Europe N.V. in the CLH report prepared and submitted by the Austrian Agency for Health and Food Safety (version 2, March 2011).
ISK Biosciences Europe N.V. is the sole notifier of fluazinam in Europe and as such has presented their view on classification and labelling of fluazinam based on the data package created during the EU review process. The table below summarizes the proposals for classification and labelling of fluazinam by the rapporteur, the Austrian Agency for Health and Food Safety and by the notifier, ISK Biosciences Europe.

<table>
<tr><td colspan="2">Current proposal by the Austrian Agency for Health and Food Safety for consideration by RAC</td><td colspan="2">Proposal by ISK Biosciences Europe for consideration by RAC</td></tr>
<tr><td>CLP Regulation</td><td>Directive 67/548/EEC (Dangerous Substances Directive; DSD)</td><td>CLP Regulation</td><td>Directive 67/548/EEC (Dangerous Substances Directive; DSD)</td></tr>
<tr><td>Cat. 4, H332
Cat. 3, H335
Cat. 2, H315
Cat. 1, H318
Cat. 1, H317
Repr. Cat. 2, H361</td><td>Xn, R20
Xi, R37
Xi, R38
Xi, R41
Xi, R43
Repr. Cat. 3, R63</td><td>Cat. 4, H332
Cat. 2, H315
Cat. 1, H318
Cat. 1, H317

aquatic</td><td>Xn, R20
Xi, R38
Xi, R41
Xi, R43

N Dangerous for</td></tr>
</table>
</td><td>Noted.
The studies mentioned in this report and commented by ISK have been reviewed in detail in the DAR and the Addenda to the DAR of fluazinam. In addition, the studies mentioned have been peer reviewed by the experts and the outcome is available in the EFSA conclusion!</td><td>Noted. These data have been assessed in the opinion.</td></tr>
</table>

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

aquatic environmental hazard acute category 1 H400: Very toxic to aquatic life aquatic environmental hazard chronic category 1 H410: Very toxic to aquatic life with long lasting effects.	N Dangerous for the Environment R50 Very toxic to aquatic organisms R53 May cause long term effects in the environment	environmental hazard acute category 1 H400: Very toxic to aquatic life aquatic environmental hazard chronic category 1 H410: Very toxic to aquatic life with long lasting effects.	the Environment R50 Very toxic to aquatic organisms R53 May cause long term effects in the environment

ISK Biosciences Europe does not propose to label fluazinam with Cat. 3, H335 and Repr. Cat. 2, H361. A rationale for non classification with Cat. 3, H335 and Repr. Cat. 2, H361 is included in Appendix I and II to this letter.
Yours sincerely,

Sarah Stiénon
Senior registration specialist
ISK Biosciences Europe N.V.

ECHA comment: The document attached Letter from ISK Biosciences Europe N.V 04/07/2011, "CLH report – Proposal for classification and labeling of the active substance fluazinam", (IBE comment fluazinam classification proposal.pdf) is copied below:

Appendix II :
Rationale for non classification Repr. Cat. 2, H361 according to Regulation (EC) No 1272/2008 (Xn, R63)
Two teratogenicity studies with fluazinam in the rat are conducted:
First study (Willoughby, 1985).
Maternal and development NOAEL: 10mg/kg bw/day

<table>
<tr><th>Date</th><th>Country/ Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
Maternal effects: decreased food consumption and weight gain
Developmental: decrease of fetal and placental weight; increased developmental variations

Repeat study (Beck, 2006).
Maternal and development NOAEL: 10mg/kg bw/day
Maternal effects: decreased food consumption and weight gain
Developmental: increased post-implantation loss; decreased fetal weight; developmental variations

In the process of the EFSA peer-review for Fluazinam toxicity, findings in the first rat teratogenicity study[1)] had triggered R63 risk phrase labelling on Fluazinam. The representative findings in the first rat developmental study can be summarized below in the table-1.

Table-1: Findings in the first rat developmental study

<table>
<tr><td>Findings / Dose (mg/kg/day)</td><td>0</td><td>10</td><td>50</td><td>250</td></tr>
<tr><td colspan="5">A. Fetal observations at necropsy</td></tr>
<tr><td>1. Large placenta</td><td>6.2 (6)*</td><td>2.6 (5)</td><td>0.7 (1)</td><td>0.0 (0)</td></tr>
<tr><td>2. Small fetus</td><td>5.8 (8)</td><td>4.4 (9)</td><td>6.6 (10)</td><td>31.5 (15)</td></tr>
<tr><td>3. Rudimentary tail</td><td>0.7 (2)</td><td>0</td><td>0.3 (1)</td><td>0</td></tr>
<tr><td>4. 'shiny' fetus – (general edema)</td><td>1 (0.4)</td><td>3 (1.1)</td><td>0</td><td>1 (0.4)</td></tr>
<tr><td>5. Bilateral cleft lip</td><td>0</td><td>0</td><td>0</td><td>0.4 (1)</td></tr>
<tr><td>6. Unilateral cleft upper jaw</td><td>0</td><td>0</td><td>0</td><td>1.9 (1)</td></tr>
<tr><td>7. Deformed palate</td><td>0</td><td>0</td><td>0</td><td>1.9 (2)</td></tr>
<tr><td>8.Edematous pup, digital anomalies, bilateral microphthalmia, heart and lung anomalies, hermaphrodite</td><td>0.4 (1)</td><td>0</td><td>0</td><td>0</td></tr>
<tr><td>9. Pale area on</td><td>0.4 (1)</td><td>0.4 (1)</td><td>0</td><td>0</td></tr>
</table>
</td><td></td><td></td></tr>
</table>

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

placental edge				
10. Amniotic membrane green	0	0	0	10.5 (2)
B. Fetal observations after free-hand serial sectioning				
11. folded retina	0	2.3 (2)	0	3.9 (2)
12. s. dilatation of lateral ventricle	3.7 (4)	0	1.4 (2)	7.8 (5)
13. Cleft palate	0	0	0	2.3 (1)
14. Hydro-ureter	5.2 (4)	3.8 (3)	2.9 (3)	3.9 (4)
15. Diaphragmatic hernia	0	0	0	3.1 (2)
16. Subcutaneous edema	16.4 (8)	13.8 (6)	12.2 (6)	20.2 (6)
C. Fetal observations at skeletal examination				
17. Absence of hyoid bone	7.1 (6)	9.3 (9)	6.1 (7)	5.1 (5)
18. Number of ribs 14/14	0.7 (1)	1.4 (2)	2.7 (4)	2.9 (3)
19. One or more ribs wavy	0.7 (1)	0	0	0
20. Incomplete ossification of one or more thoracic vertebrate center	34.3 (20)	47.9 (18)	46.3 (20)	55.1 (20)

*: % incidence (number of litters)

In the findings from the first rat developmental study, the most serious incidences were clefts of the lip or palate, diaphragmatic hernia, supernumerary ribs and anomalies of the thoracic _abelling bodies being a factor to have triggered R63 for Fluazinam during RMS and EFSA evaluation. But besides them, hemorrhage and edema were remarkably observed in all groups without dose-response reactions. Incomplete ossifications in various regions were reported to have slightly increased for the highest dose group compared with other groups, but this finding seemed to be related to the growth retardation due to the primary dam's toxicity from 14-day consecutive gavage administrations.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Due to scientific progress, the first study was conducted in 1984-1985, new insights on the use of the vehicle for administration of test substance justified the need to repeat the study. One of the differences between the first and repeat study is the vehicle used in both studies. Namely, the first study used corn oil, while the second study used CMC-Na aqueous solution. The absorption rate is almost at the same level for both vehicles in the rat [3)]. However the use of corn-oil can create undesirable effects which could be incorrectly attributed to the test substance. Dr. M. Sato has recently reported the influence of corn oil and diet on reproduction and kidneys in female SD rats at 2000 into TOXICOLOGY SCIENCES [4)]. The dams which were administered by corn oil had shown severe lesions in the proximal tubular epithelium of the kidney which is the critical area to re-absorb the secreted urea. Therefore, such serious finding in the kidney can be a cause for the disorder of the body water control, which could result in general edema elsewhere in the body. The kidneys of the dams in the first rat developmental study had not examined histopathologically but it can suppose the dams should have similar kidney changes due to the corn oil from 10 time consecutive administrations of 10 ml/kg/day. The edematous findings in the fetuses might be related to a failure for the water control valance by the dam kidney. Actually several results in the control group of the first study had shown unusual findings such as large placenta, dilatation of the lateral ventricle, and hydro-ureter etc. They might be related to the water valance disorder due to the dam's renal effects by corn oil administrations. The quality of the corn oil used in the first study was not described at all in the study report. If the corn oil used were derived from yellow maize, then it could contain some amount of carotinoids which is precursor of Vitamin A. Historically many reports have been published highlighting the adverse effects of retinol and retinoid in the rat developmental studies [5-7)]. They have reported increases of cleft palate, diaphragmatic hernia, and small fetuses etc. Another similar phenomenon has been reported for the teratogenicity of palm oil due to the impurity of carotene in it [8)]. Although it can only be supposes that the vehicle is a contributing factor for the findings in the first study, the second study has clearly shown that fluazinam itself does not induce adverse effects in the rats. Usually water based vehicle should be considered as the first choice in regulatory toxicological studies. If fluazinam would have adverse teratological effects to the fetuses, then, the repeat study should have reflected the same adverse effects such as diaphragmatic hernia and cleft lips and palates but the effects observed in the second study are not confirmatory for the effects observed from the first		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		study. In addition higher doses were administered in the repeat study in order to try to clarify on the effects. Based on the repeat rat developmental study carefully, Fluazinam does not need to have the serious R63 phrase on the label because no increased sensitivity in fetuses compared with the dam toxic findings in the liver. A guiding principle of whether a compound has true teratogenic potential is that findings should be reproducible when tested under the same, or similar, experimental conditions. This principle can be invoked where data have shown ambiguous or equivocal findings or where different opinions on interpretation have been expressed. In these circumstances, treatment at a higher dosage may elicit a stronger response. Upon availability of two teratology studies with fluazinam in rats, the data obtained have been reviewed by the study director of the first study. His review and conclusions are presented in a separate paper included to this appendix[9)]. On the basis of the repeat study performed at dosages of 0, 10, 50 and 300 mg/kg bw/day, together with a re-evaluation of the data from the original study, there seems to be no justification for an R 63 labeling to be applied due to teratogenic potential. M. Nomura, Ph. D. Group Leader of Regulatory affairs and safety groups Biosciences HQ at Osaka in Japan Ishihara Sangyo Kaisha, Ltd. References: 1. Willoughby, C. R., Tesh, J. M., et al, 1985, B-1216: Teratology study in the rat, LSR Report Number: 84/ISK047/606, amended final report number: 91/ISK047/0820 2. Beck, M.J., 2006, A prenatal developmental toxicity study of technical fluazinam in rats, WIL Research Laboratories Report Number WIL-282006 3. Marciniszyn J., 1995, Study of the billiary excretion of radiolabel following oral administration (phenyl – 14C) –IKF-1216 to male Sprague-Dawley rats, Ricerca Report No. 5318-92-0321-AM-001 4. Sato, M., Wada K., et al, 2000, Influence of Corn Oil and Diet on Reproduction and the Kidney in Female Sprague-Dawley Rats, Toxicological Sciences Vol. 56, 156-164 5. Padmanabhan R. and Ahmed I., 1997, Retinoic acid-induced asymmetric craniofacial growth and cleft palate in the TO mouse fetus, Reprod Toxicol Vol. 11 (6), 843-860		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		6. Beurskens L. W., Tibboel D., et al., 2010, Retinol status of newborn infants is associated with congenital diaphragmatic hernia, Pediatrics Vol. 126, 712-720 7. Kling D., E. and Schnitzer J., J., 2007, Vitamin A deficiency (VAD), teratogenic, and surgical models of congenital diaphragmatic hernia (CDH), Am. J. Med. Genet. C. Semin. Med. Genet, Vol. 15 145C (2), 139-157 8. Singh, J. D., 1979, Teratogenicity of palm oil in albino rats, Cong. Anom., Vol. 19, 125-128 9. Bottomley, A.M., and Willoughby, C.R., 2006, Fluazinam (B-1216) Overview of Embryo-fetal studies in the CD rat, HLS Document Number: ISK0277/060106 *ECHA comment: The document attached Ishihara Sangyo Kaisha, Ltd., 2006, Final Report – A Prenatal Developmental Toxicity Study of Technical Fluazinam in Rats, WIL-282006, Japan. (Reference 2.pdf) is copied below:* FINAL REPORT STUDY TITLE A PRENATAL DEVELOPMENTAL TOXICITY STUDY OF TECHNICAL FLUAZINAM IN RATS STUDY NUMBER WIL-282006 DATA REQUIREMENTS OPPTS Guideline 870.3700 OECD Guideline 414 STUDY DIRECTOR Melissa J. Beck, PhD STUDY INITIATION DATE 7 November 2005 STUDY COMPLETION DATE 31 March 2006 REPORT AMENDED ON 4 April 2006 PERFORMING LABORATORY WIL Research Laboratories, LLC 1407 George Road Ashland, OH 44805-9281 SPONSOR		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Ishihara Sangyo Kaisha, Ltd. 3-15, 1-chome Edobori, Nishi-ku Osaka 550-0002, JAPAN STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) 1(A), (B) or (C). Sponsor: Ishihara Sangyo Kaisha, Ltd. 3-15, 1-chome Edobori, Nishi-ku Osaka 550-0002, JAPAN Sponsor's Agent: _____ _____ GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT This study, designated WIL-282006, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 160), 16 October 1989; the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792), 18 September 1989; the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice [C(97) 186/Final], 26 November 1997; the standard operating procedures of WIL Research Laboratories, LLC and the protocol as approved by the sponsor. This study is subject to the applicable regulations of the OECD Guidelines for the Testing of Chemicals Guideline 4 14, Prenatal Developmental Toxicity Study, January 200 1 and the United States EPA Health Effects Test Guidelines OPPTS 870.3700, Prenatal Developmental Toxicity Study, August 1998. Melissa J. Beck, PhD Assistant Director, Neuroscience Study Director D. Nakio, DVM,MS Sponsor Representative Date ApplicantBubmitter Date		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		FLAGGING STATEMENT I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria. _____ _____ TABLE OF CONTENTS Page Statement Of No Data Confidentiality Claims ..... 2 Good Laboratory Practice Compliance Statement ..... 3 Flagging Statement..... 4 Table Of Contents ..... 5 Index Of Tables ..... 8 Index Of Appendices..... 10 1. Summary ..... 11 1.1. Objective ..... 11 1.2. Study Design ..... 11 1.3. Results ..... 11 1.4. Conclusions ..... 13 2. Introduction ..... 14 2.1. General Study Information..... 14 2.2. Key Study Dates..... 14 3. Study Design ..... 15 4. Experimental Procedures – Materials And Methods ..... 16 4.1. Test Article And Vehicle..... 16 4.1.1. Test Article Identification ..... 16 4.1.2. Vehicle Identification..... 16 4.1.3. Preparation ..... 16 4.1.4. Sampling And Analyses ..... 18 4.2. Test System ..... 18 4.3. Organization Of Test Groups, Dosage Levels And Treatment Regimen ..... 19 4.4. Animal Receipt And Acclimation ..... 19 4.5. Animal Housing ..... 20 4.6. Diet, Drinking Water And Maintenance 20		

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		C. Animal Room Environmental Conditions.....338 D. WIL Developmental Historical Control Data Version 3.3 [Crl:CD(SD) Rat] .....344 E. Study Protocol.....371 F. Report Amendment I .....394 1. SUMMARY 1.1. OBJECTIVE The objective of this study was to determine the potential of the test article, technical fluazinam, to induce developmental toxicity after maternal exposure during the critical period of organogenesis, to characterize maternal toxicity at the exposure levels tested and to determine a NOAEL (no-observed-adverse-effect level) for maternal toxicity and developmental toxicity. 1.2. STUDY DESIGN The test article, technical fluazinam, in the vehicle, 0.5% carboxymethylcellulose sodium (CMC-Na), was administered orally by gavage to 3 groups of 25 bred female Crl:CD(SD) rats once daily from gestation days 6 through 19. Dosage levels were 10, 50 and 300 mg/kg/day administered at a dosage volume of 5 mL/kg. A concurrent control group composed of 25 bred females received the vehicle (0.5% CMC-Na) on a comparable regimen. All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights and food consumption were recorded at appropriate intervals. On gestation day 20, a laparohysterectomy was performed on each female. The contents of the thoracic, abdominal and pelvic cavities were examined, and the liver was weighed. The uteri, placentae and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations and corpora lutea and placental weights were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed, sexed and examined for external, visceral and skeletal malformations and developmental variations. 1.3. RESULTS All maternal animals survived to the scheduled necropsy on gestation day 20. There were no test article-related clinical findings observed at the daily examinations, and no significant clinical observations were noted 1 hour following dose administration. A mean body weight loss at 300 mg/kg/day and a reduced mean body weight gain at 50 mg/kg/day with corresponding reductions in food consumption were observed early in gestation (gestation days 6-9); the differences were attributed to administration of the test article. Mean body weight gains in the 50 and 300 mg/kg/day groups and mean food		

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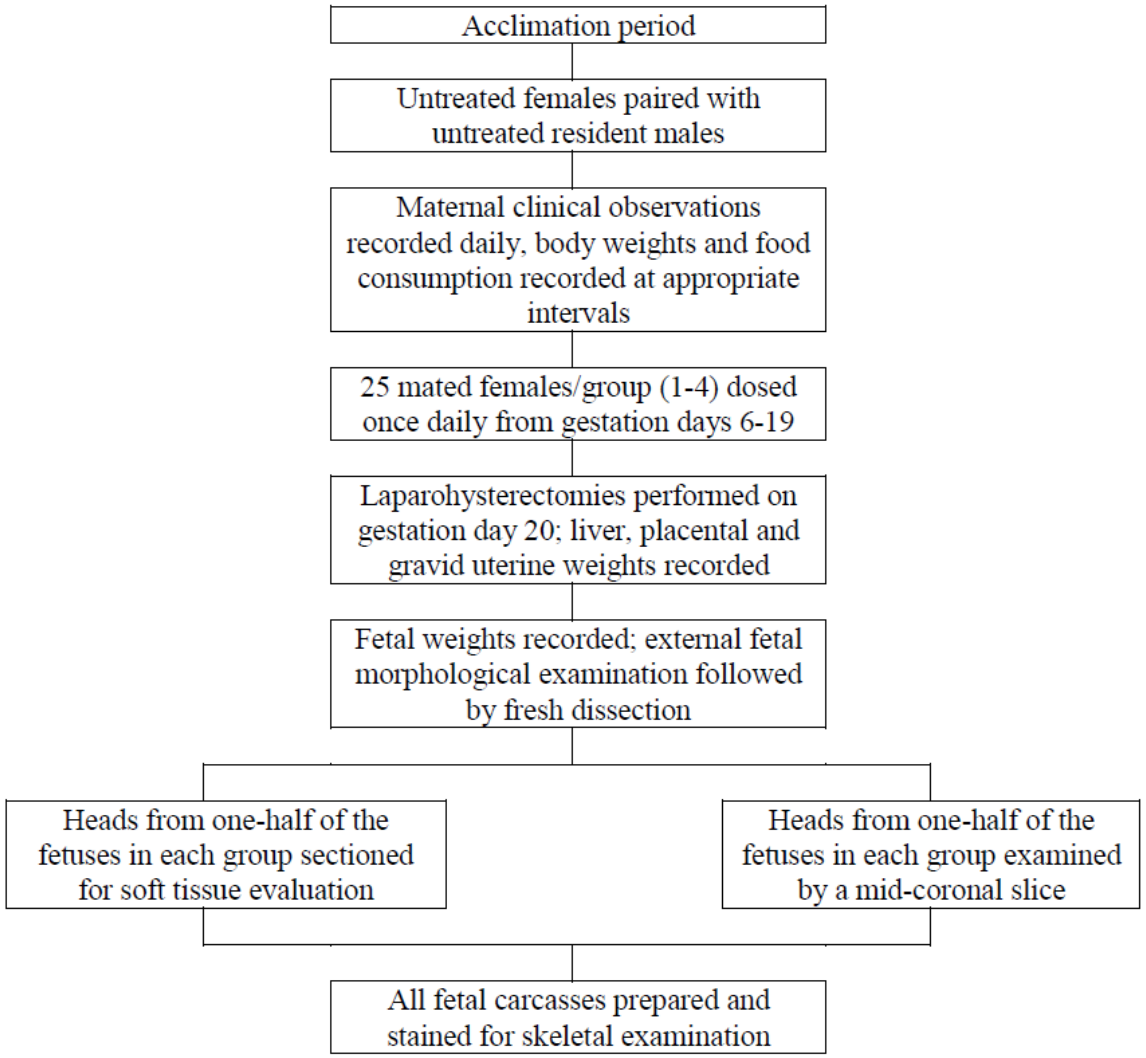
		consumption in the 50 mg/kg/day group were generally similar to the control group values for the remainder of the treatment period, with the following exception. Mean body weight gain in the 300 mg/kg/day group was reduced compared to the control value during gestation days 15-20. However, this difference was primarily attributed to the decreased mean gravid uterine weight that corresponded to a decrease in the mean number of viable fetuses and reduced mean fetal weights noted in this group. Food consumption in the 300 mg/kg/day group continued to be lower than control values for the remainder of the treatment period, and mean body weights in this group were lower than the control group values from gestation days 8 to 20. Mean net body weight and net body weight gain in the 300 mg/kg/day group were reduced compared to the control group values. Mean maternal body weights, body weight gains and food consumption in the 10 mg/kg/day group and net body weights, net body weight gains and gravid uterine weights in the 10 and 50 mg/kg/day groups were unaffected by test article administration. At the time of necropsy, mean liver weights were increased in a dose-related manner in the 10, 50 and 300 mg/kg/day groups compared to the control group. Although test article-related, the increased mean liver weights were not considered adverse because previous data demonstrate that centrilobular hepatocellular hypertrophy is reversible after cessation of treatment (Broadmeadow, 1991). No test article-related internal findings were observed at any dosage level. Test article-related effects on intrauterine growth and/or survival were noted in the 50 and 300 mg/kg/day groups. The mean number and litter proportion of viable fetuses in the 300 mg/kg/day group were lower than the control group values as a result of an increase in the mean litter proportion of postimplantation loss (primarily early resorptions). Mean fetal body weights in the 50 and 300 mg/kg/day groups were reduced compared to the control group. Corresponding visceral and skeletal developmental variations (increased mean litter proportions and/or occurrences of renal papilla(e) not developed [or underdeveloped] and/or distended ureter(s), and increased mean litter proportions of 27 presacral vertebrae and reduced ossification of the skull, unossified sternebrae nos. 5 and/or 6, unossified sternebrae nos. 1, 2, 3 and/or 4 and/or reduced ossification of the vertebral arches and/or a decreased mean litter proportion of ossified cervical centrum no. 1) were seen at the same dosage levels. Intrauterine growth and survival and fetal morphology at 10 mg/kg/day were unaffected by test article administration. 1.4. CONCLUSIONS Based on decreased mean maternal body weight gains and food consumption at 50 and		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		300 mg/kg/day, a dosage level of 10 mg/kg/day was considered to be the no-observed-adverse-effect level (NOAEL) for maternal systemic toxicity. Based on increased mean litter proportions of postimplantation loss at 300 mg/kg/day, decreased mean fetal body weights at 50 and 300 mg/kg/day, and increased mean litter proportions of several developmental variations at 50 and 300 mg/kg/day, a dosage level of 10 mg/kg/day was considered to be the NOAEL for embryo/fetal developmental toxicity when technical fluazinam was administered orally by gavage to pregnant Crl:CD(SD) rats. The test article-related developmental variations occurred in conjunction with maternal toxicity at 50 and 300 mg/kg/day. However, the developmental variations were generally considered secondary to growth retardation. In conclusion, technical fluazinam did not exhibit any evidence of teratogenicity in fetuses in this study. 2. INTRODUCTION 2.1. GENERAL STUDY INFORMATION This report presents the data from "A Prenatal Developmental Toxicity Study of Technical Fluazinam in Rats". Due to software spacing constraints, the study title is presented as "A Prenatal Dev. Tox. Study of Technical Fluazinam in Rats" on the report tables. The following computer protocol was used for data collection during the study: Computer Protocol Type of Data Collected WIL-282006.....Main study data 2.2. KEY STUDY DATES Date(s) Event(s) 15 November 2005 .....Experimental starting date (animal receipt) 29 November 2005 .....First gestation day 0 5 December 2005.....Experimental start date (first day of test article administration) 5-21 December 2005.....Test article administration 22 December 2005.....Last laparohysterectomy 9 February 2006 .....Experimental termination/completion date (last fetal skeletal examination)		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		3. STUDY DESIGN  4. EXPERIMENTAL PROCEDURES – MATERIALS AND METHODS		

Respiratory sensitisation

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	Finland / Member State	In section 4.6.2 it is stated that based on data from the acute inhalative studies, it can be concluded that fluazinam is not a respiratory sensitizer. According to CLP substances shall be classified as respiratory sensitisers (Category 1) in accordance with the following criteria: (a) if there is evidence in humans that the substance can lead to specific respiratory hypersensitivity and/or b) if there are positive results from an appropriate animal test. Our opinion is that both possibilities should be considered in justifications for this end point. Also other relevant data from animal tests should be considered (e.g., repeated dose studies by inhalation route if available) and evidence in humans. For your information, we found article which discusses occupational asthma caused by sensitisation to powdered fungicides fluazinam and chlorothalonil (Draper, A, P Cullinan, C Campbell, et al. 2003. Occup Environ Med 60: 76-77). Furthermore, because the substance is already classified as a skin sensitizer (positive in M-K test and Buehler test) it is important to consider all available data. It is possible that the substance can induce reactions in the airways as well as in the skin.	Noted.	Noted.
01/07/2011	Germany / Member State	Comment on behalf of the German CA: We disagree that respiratory sensitisation can be excluded for fluazinam based on data from acute inhalative studies. These studies do not allow for this conclusion because they do not comprise an induction and challenge phase. Therefore, we kindly ask to change the entry in table 3 (see Part A, chapter 1.3: "Proposed classification according to the CLP Regulation") concerning the reason for non-classification as respiratory sensitiser (see 3.4.) from "conclusive, but not sufficient for classification" to "Data lacking".	"Conclusive, but not sufficient for classification" will be changed to "Data lacking" in revised CLH report as part of the RCOM, see Annex 2.2	Ok. Done.
04/07/2011	United Kingdom / UK Competent Authority / MSCA	P38, section 4.6.2 It is not appropriate to use those data obtained from acute inhalation studies to assess whether a substance is a respiratory sensitiser, therefore this statement is invalid. However we agree that fluazinam should not be classified for this end point, but based on a lack of data.	Will be changed in revised CLH report as part of the RCOM, see Annex 2.2	Ok. Done.
04/07/2011	Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer	p.30 comparison with criteria and conclusions on classification and labelling; company does not agree with R37 classification. Detailed raisoning why fluazinam should not be classified R37 is explained in attached documents. *ECHA comment: The document attached Letter from ISK Biosciences Europe N.V 04/07/2011, "CLH report – Proposal for classification and labeling of the active substance fluazinam", (IBE comment fluazinam classification proposal.pdf) is copied below:*	The studies mentioned in this report and commented by ISK have been reviewed in detail in the DAR and the Addenda to	Noted.

<table>
<tr><th>Date</th><th>Country / Organisation / MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
Dear Sir, Madam,

Subject: CLH report – Proposal for classification and labelling of the active substance fluazinam
This document is aimed to address point 2.4.1 self-classification and labelling proposal based on the CLP Regulation criteria by the notifier ISK Biosciences Europe N.V. in the CLH report prepared and submitted by the Austrian Agency for Health and Food Safety (version 2, March 2011).

ISK Biosciences Europe N.V. is the sole notifier of fluazinam in Europe and as such has presented their view on classification and labelling of fluazinam based on the data package created during the EU review process. The table below summarizes the proposals for classification and labelling of fluazinam by the rapporteur, the Austrian Agency for Health and Food Safety and by the notifier, ISK Biosciences Europe.

<table>
<tr><td colspan="2">Current proposal by the Austrian Agency for Health and Food Safety for consideration by RAC</td><td colspan="2">Proposal by ISK Biosciences Europe for consideration by RAC</td></tr>
<tr><td>CLP Regulation</td><td>Directive 67/548/EEC (Dangerous Substances Directive; DSD)</td><td>CLP Regulation</td><td>Directive 67/548/EEC (Dangerous Substances Directive; DSD)</td></tr>
<tr><td>Cat. 4, H332
Cat. 3, H335
Cat. 2, H315
Cat. 1, H318
Cat. 1, H317
Repr. Cat. 2, H361
aquatic environmental hazard acute category 1
H400: Very toxic to aquatic life

aquatic environmental hazard chronic</td><td>Xn, R20
Xi, R37
Xi, R38
Xi, R41
Xi, R43
Repr. Cat. 3, R63
N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment</td><td>Cat. 4, H332
Cat. 2, H315
Cat. 1, H318
Cat. 1, H317

aquatic environmental hazard acute category 1
H400: Very toxic to aquatic life

aquatic environmental hazard chronic category 1</td><td>Xn, R20
Xi, R38
Xi, R41
Xi, R43

N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment</td></tr>
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</td><td>the DAR of fluazinam.</td><td></td></tr>
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Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment

category 1 H410: Very toxic to aquatic life with long lasting effects.		H410: Very toxic to aquatic life with long lasting effects.	

ISK Biosciences Europe does not propose to label fluazinam with Cat. 3, H335 and Repr. Cat. 2, H361. A rationale for non classification with Cat. 3, H335 and Repr. Cat. 2, H361 is included in Appendix I and II to this letter.
Yours sincerely,

Sarah Stiénon
Senior registration specialist
ISK Biosciences Europe N.V.

Appendix I :
Rationale for non classification Cat. 3, H335 according to Regulation (EC) No 1272/2008 (Xi, R37)
Two acute inhalation studies with fluazinam are conducted:
First study (Tobeta, 1988).
LC50 m: 0.463mg/l air
LC50 f : 0.476 mg/l air
(Study design: 4h, whole body exposure)
Signs of hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea were observed in an acute inhalative toxicity study.

Repeat study (Kirkpatrick, 2006).
m/f > 1.1 mg/l air
(Study design: 4h, nose only exposure)
Dark red discoloration of the lungs.

In the first acute inhalation study the LC_{50} was 0.463 mg/l for males and 0.476 mg/l for females (Study design: 4h, whole body exposure). It was sceintifically justified to repeat this study because of two main shortcomings which can be consedered to be scientifically not correct with current knowledge: the study design and the vehicle used.

(1) Vehicle: in the first acute inhalation test, polyethylene glycol 400 was used as the vehicle for

Date	Country / Organisation / MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		administration of fluazinam by oral route. This was done because with techniques available at time of study conduct (1987) the preparation of dust aerosol with suitable fineness was not possible. Fluazinam is completely soluble in polyethylene glycol 400, hence the exposure results in the study will be different from actual exposure and likely to produce false positive results. Generally speaking, chemicals show higher toxicity in solution form than in solid form, because of higher contact and intake to organ at molecular level. Techniques for production of high concentration of powder in air have greatly improved in the mean time and it was reasonable to test the acute inhalation with improved techniques. In the repeat study fluazinam was administered as a dust aerosol which corresponds to the actual exposure. (2) Study design: the study design in the first study was 'whole body exposure' which includes oral, dermal and inhalation route, where in the repeat study the exposure design is 'snout-only' in accordance with current OECD guideline for the testing of chemicals no. 403. In the repeat study oral or dermal route are excluded such that the observations made are fully representative of oral exposure only and the inhalation LC_{50} result is a more correct estimation of the acute inhalation properties of fluazinam. In the repeat study the endpoint obtained is LC_{50} > 1.1 mg/l air and demonstrates that the first study provided in an unrealistic exposure. The improved toxicological result can be attributed to the vehicle effect and the improved study design to exclude other than inhalation exposure. During the EU evaluation this rationale was accepted, at the meeting PRAPeR 29 (0519 – 20 07.2007) experts have agreed that the repeat study was more reliable to base the classification for acute toxicity by inhalation upon. The experts also concluded that the value LC50 >1.1 mg/L was not the highest dose technically achievable and according to this, the proposed classification was Xn, R20 Harmful by inhalation corresponding to Cat. 4, H332 under the CLP regulation. This was confirmed in the Peer Review report prepared by EFSA[1]. It should be noted that this is also the conclusions for the acute toxicity under point 4.2.4 of the CLH report. For a reason not justified he proposal made under point 1.3 is different. ISK Biosciences Europe N.V. confirms to agree with the conclusions made by PRAPeR 29 and is of the opinion that classification Xn, R20 Harmful by inhalation corresponding to Cat. 4, H332 under the CLP regulation is correct. [1] EFSA Scientific Report (2008) 137, 1-87, Conclusion on the peer review of fluazinam		

Other hazards and endpoints

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	Spain / Member State	We are not in agreement with the Austrian environmental proposal of the deletion of the Chronic M factor of 10 based on the rapid degradation of Fluazinam our recommendation is to keep it due to the lack of information on the stable metabolite AMPA.	We agree with the Spanish authorities. Acute aquatic toxicity data for AMPA were available and will be amended in revised CLH report as part of the RCOM, see Annex 2.2; Chronic M factor of 10 was proposed	In the CLH report Fluazinam is considered not rapidly degradable and a Chronic M-factor of 10 is proposed. We agree with this proposal.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	Belgium / Member State	Based on the results of the aquatic toxicity test on the most sensitive species (Acute toxicity : 96hEC50fish = 0.036mg/l, chronic toxicity : NOEC fishF0growth, F1 survival = 0.0029mg/l) , the fact that the substance is not rapidly biodegradable (not readily biodegradable within 28days, Aquatic water/sediment test : primary degradation with DT50<16d, but low ultimate degradation)and that the substance shows potential to bioaccumulate (BCF between 906 and 1090), it is justified to classify, following the classification criteria of the 2nd ATP, as Aquatic acute cat 1, H400 and Aquatic chronic 1, H410.	Noted will be amended in revised CLH report as part of the RCOM, see Annex 2.2	Ok.
		In view of the proposed classification and the toxicity band for acute toxicity between 0.01 mg/l and 0.1mg/l, an M-factor for acute toxicity of 10 could be assigned, and an M-factor for chronic toxicity of 10 (non-rapidly degradable substance and toxicity band between 0.001mg/l and 0.01 mg/l).	Noted	Ok.
		Based on the classification and labelling criteria in accordance with dir. 67/548/EEC, Fluazinam should be classified as N, R50/53.	Noted	Ok.
		In conclusion : we agree with the proposed environmental classification by the Austrian MSCA.	will be amended in revised CLH report as part of the RCOM, see Annex 2.2	
		Some editorial or/and minor comments: It would be useful to refer already in each section "summary and discussion" of the environmental endpoints to the DSD and CLP criteria.		
		5.1.2.3. Aerobic/sediment study The DT50-values are indeed <16days and some major metabolites are formed by hydrolysis or reduction. Mineralization to CO2 is very low. However data on primary degradation can only be used for classification if it is demonstrated that the degradation products are not classified for the environment.	Acute aquatic toxicity data for AMPA were available and will be amended in revised CLH report (Annex 2.2) Chronic M factor of 10 was proposed	In the CLH report Fluazinam is considered not rapidly degradable and a Chronic M-factor of 10 is proposed. We agree with this proposal.
		5.3.2 Summary and discussion of aquatic bioaccumulation	will be included in revised CLH report as part of the RCOM, see Annex 2.2	
		For substances with high lipophilicity (log Kow >3): BCFwhole fish should be corrected in relation to lipid content of test fish (in function of total wet weight of fish).	In DAR BCF was determined only for viscera and fillet, but was not	The information on lipid content was not available in the CLH report.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
04/07/2011	Finland / Member State	Skin sensitation: Classification for skin sensitation should follow the differentiation to the hazard subcategories 1A or 1B according to the 2nd ATP of CLP regulation. Skin Irritation In section 4.4.1 the classification proposal is based on three weeks repeated dose toxicity instead of the pre-sented 4 hours dermal irritation study. Please, add justification and cross-reference to the three weeks study. Environment: In Section 1.3 specific concentration limits for N, R50-53 classification should be given. In Table 3 and 4 it is unclear what is meant with current classification. At the end of Table 3 labeling is mentioned. When the substance is classified both with H400 and H410, only H410 is cited in the labelling. Concerning ready/rapid degradability, the conclusion for classification should be the same. You should use water/sediment study results, when available, also when classifying according to Directive 67/548/EEC. We disagree with the conclusion that the substance would be rapidly degradable. The half-lives are less than 16 days but mineralization is very low. There seems to be no information on the classifiability of the degradation products which would be the only way to conclude rapid degradability from the tests described. If this information exists it should be clearly considered when discussing comparison with the criteria. Our conclusion based on the given data would be that the substance is not readily/rapidly degradable. Consequently the M-factor for long term hazard based on the lowest NOEC of 0.0029 mg/l should be 10. Please also specify that you are using the 2nd ATP criteria of the CLP regulation for environmental classification.	Will be corrected in revised CLH report as part of the RCOM, see Annex 2.2 Will be amended in revised CLH report (Annex 2.2) Will be considered in revised CLH report(Annex 2.2) Will be amended in revised CLH report (Annex 2.2) Please refer to revised CLH report which is part of the RCOM, Point 5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4	It has been modified. It has been modified. Corrected in the CLH report, but the SCLs have not been revised. Corrected SCLs are included in the opinion.

<table>
<tr><th>Date</th><th>Country/ Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td>01/07/2011</td><td>Sweden / Member State</td><td>Skin irritation
KemI supports classification for skin irritation according to Dir. 67/548/EEC and for skin irritation cat.2 according to Reg. 1272/2008. Even though the dermal irritation study in rabbits demonstrates slight irritation but doesn't fulfil criteria for classification as a skin irritant. The repeated dermal administration of 10, 100 and 1000mg/kg in rats revealed macro- as well as microscopic changes in the skin. The doses correspond to approximately 0.1, 1 and 10 mg/cm2; to be compared to the dose used in TG 404, approximately 80 mg/cm2 (see IR/CSA, Figure R.7.2-2, footnote d). Additionally, rat skin is less sensitive than rabbit skin.

Eye irritation
In a rabbit study corneal, iridal and conjunctival effects persisted through day 21; thus classification for serious eye damage according to Dir. 67/548/EEC and for serious eye damage cat.1 according to Reg. 1272/2008 is supported.
Respiratory irritation
In the acute inhalation study by Tobeta (1988) pulmonary emphysema was identified, being an irreversible effect. The relevance of this effect may need to be further elucidated in the original report as respiratory irritation is normally referred to as transient effects. Other reported respiratory effects by Tobeta (1988) and Kirkpatrick (2006) appear to be transient and signs of respiratory irritation. Classification for respiratory irritation according to Dir. 67/548/EEC and for STOT-SE (respiratory irritation) cat.3 according to Reg. 1272/2008 is supported.

Skin sensitization
One Magnusson & Kligman test and one Buehler test reported. Both turned out positive. Pre-screening tests were performed in both cases to avoid testing with irritating concentrations. Classification for skin sensitization according to Dir. 67/548/EEC and for skin sensitization cat.1 according to Reg. 1272/2008 is supported.</td><td>Noted

Noted

Noted

Noted</td><td>Noted.
It is discussed in the opinion

Noted.

Noted.

Noted.</td></tr>
<tr><td>09/06/2011</td><td>Switzerland / Makhteshim Agan Holding B.V. (on behalf of Makhteshim Chemical Works Ltd.) / Company-Manufacturer</td><td>see attachment: zip folder containing "table 1" and the respective 6 study reports referred to

ECHA comment: The document attached Makhteshim Chemical Works, Summary of acute toxicity of Fluazinam technical, Table 1. (table 1.docx) is copied below:

Table 1

Summary of acute toxicity of Fluazinam technical (Makhteshim Chemical Works)

<table><tr><th>Type of study</th><th>Species</th><th>Result</th><th>Reference</th></tr></table></td><td>Studies will be presented in revised CLH report as part of the RCOM, see Annex 2.2</td><td>Done.</td></tr>
</table>

<table>
<tr><th>Date</th><th>Country/ Organisation/ MSCA</th><th>Comment</th><th>Dossier submitter's response to comment</th><th>RAC's response to comment</th></tr>
<tr><td></td><td></td><td>
<table>
<tr><td>Acute oral LD_{50}</td><td>Rat,
CD® (Crl: CD®)</td><td>LD_{50} > 2000 mg/kg bw</td><td>Chevalier, F (2006); 19774/06, Sponsor report no. R-20269</td></tr>
<tr><td>Acute dermal LD_{50}</td><td>Rat,
CD® (Crl: CD®)</td><td>LD_{50} > 2000 mg/kg bw</td><td>Chevalier, F. (2006); 19775/06, Sponsor report no. R-20270</td></tr>
<tr><td>Acute inhalation LC_{50} (4h)</td><td>Rat,
HsdRCCHan™ : WIST</td><td>LC_{50} inhalation rat > mean achieved atmosphere concentration of 4.82 mg/L</td><td>Griffiths, D. R. (2009), 0306/0391, Sponsor report no. R-24975</td></tr>
<tr><td>Acute skin irritation</td><td>Rabbit,
Himalayan</td><td>non-irritating</td><td>Leuschner, J. (2006); 19777/06, Sponsor report no. R-20272</td></tr>
<tr><td>Acute eye irritation</td><td>Rabbit,
Himalayan</td><td>non-irritating</td><td>Leuschner J. (2006); 19778/06, Sponsor report no. R-20273</td></tr>
<tr><td>Skin sensitisation – Magnusson & Kligman test</td><td>Guinea pig,
Dunkin-Hartley</td><td>non-sensitising</td><td>Chevalier, F. (2006); 19779/06, Sponsor report no. R-20274</td></tr>
</table>

ECHA comment: View document attached Griffiths D R, 2009, MCW 465 tech: Acute Inhalation Toxicity (Nose Only) Study in the Rat, Project Number 0306/0391, Sponsor Number R – 24975. (R-24975.pdf).

ECHA comment: View document attached Leuschner P, 2006, Acute Dermal Toxicity Study of MCW 465 in Rats, Laboratory of Pharmacology and Toxicology, Sponsor No R-2027, Gernamy (R-20270.pdf).

ECHA comment: View document attached Leuschner P, 2006, Acute Oral Toxicity Study of MCW 465 in Rats, Laboratory of Pharmacology and Toxicology, Sponsor No R-20269, Germany (R-20269.pdf).

ECHA comment: View document attached Leuschner P, 2006, Acute Dermal Irritation/Corrosion Test (Patch Test) of MCW 465 in Rabbits, Laboratory of Pharmacology and Toxicology, Sponsor No R-20272, Germany (R-20272.pdf).

ECHA comment: View document attached Leuschner P, 2006, Acute Eye Irritation/Corrosion Test of MCW 465 in Rabbits, Laboratory of Pharmacology and Toxicology, Sponsor No R-20273, Germany (R-20273.pdf).

ECHA comment: View document attached Leuschner P, 2006, Examination of MCW 465 in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test), Laboratory of Pharmacology
</td><td></td><td></td></tr>
</table>

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		and Toxicology, Sponsor No R-20274, Germany (R-20274.pdf).		
29/06/2011	Netherlands / RIVM / National Authority	Acute toxicity: In one of the acute oral toxicity studies in rats ataxia is observed at 5000 mg/kg bw (only dose tested). Exact details on the severity of the ataxia are not included in the CLH dossier (including at what dose ataxia was observed), but ataxia (when transient) fulfills the CLP criteria for classification as STOT-SE Cat 3; H336. If fluazinam is used as dust aerosol and not as solved aerosol, we agree that the second inhalation study (nose only) is more relevant for the classification of the substance. However, since in this study the LC50 is > 1.1 mg/L, the criteria for classification (both CLP and DSD) are not fulfilled (it cannot be concluded from this study that the exact LC50 will be below 5 mg/L) and the substance should therefore not be classified for acute inhalation toxicity. It is not clear why no higher concentrations of fluazinam were tested in the snout only study. Was 1.1 mg/L the highest attainable concentration? Since transient effects on the respiratory system are observed in the second inhalation study, we do agree with the classification for STOT-SE Cat 3; H335 or Xi; R37.	Ataxia was observed at a dose level of 5000 mg/kg bw (only dose tested) in this study. In the repeat inhalation toxicity study (*Kirkpatrick, 2006*) the endpoint obtained is LC_{50} > 1.1 mg/l air and demonstrates that the first study provided in an unrealistic exposure. The improved toxicological result can be attributed to the vehicle effect and the improved study design to exclude other than inhalation exposure. During the EU evaluation this rationale was accepted, at the meeting PRAPeR	This is discussed in the opinion.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			29 (0519 – 20 07.2007) experts have agreed that the repeat study was more reliable to base the classification for acute toxicity by inhalation upon. The experts also concluded that the value LC50 >1.1 mg/L was not the highest dose technically achievable and according to this, the proposed classification was Xn, R20 Harmful by inhalation corresponding to Cat. 4, H332 under the CLP regulation. This was confirmed in the Peer Review report prepared by EFSA	
		Irritation: Results on oedema and eschar formation in the skin irritation study are not included in the CLH report.	Noted;	Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Based on the observed erythema in the skin irritation study (mean value for erythema at 24, 48 and 72 h is 0.8, reversible in 14 days) the criteria of CLP (>2.3) or DSD (2) for classification as skin irritant are not fulfilled. The fact that skin effects are observed in a dermal 21 days repeated dose toxicity study is not enough for classification: 1) the duration of exposure in the repeated dose study (6h/day for 21 days) is much longer than the duration a skin irritation study (4 hours) and 2) it is not known when the first signs of skin irritation are observed in the 21 days study. The observed effects could very well be the result of sensitization (fluazinam is proposed to be classified for skin sensitization!). We therefore do not agree with the proposed classification as Skin irrit 2; H315 or Xi; R38.	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 in the 21 day study.	
		We agree with the proposed classification for eye irritation (Eye dam cat 1; H318 / Xi; R41).	Noted	Noted.
		Sensitisation: It is conclude from the M&K test that fluazinam causes delayed contact hypersensitivity. However, although indeed 30% of the test animals shows a positive response (slight-moderate erythema), also 25% of the control animals shows a positive response. Therefore, the net positive effect is only 5%, which is not enough to conclude the substance to be a skin sensitizer. Please adapt the conclusion. We agree with the proposal to classify fluazinam for skin sensitization (based on the second study). However, according to the new criteria of CLP (2nd ATP), a subcategory should be added to the classification as skin sensitizer.	Subcategory will be added in revised CLH report as part of the RCOM, see Annex 2.2	Done.
		With regard to respiratory sensitization, it is concluded in 4.6.2 of the CLH dossier that 'based on the data from the acute inhalative studies, it can be concluded that fluazinam is not a respiratory sensitizer'. This cannot be concluded from acute studies. Please replace this comment by 'no data available'.	"Conclusive, but not sufficient for classification" will be changed to "Data lacking" in the revised CLH report as part of the	Done.

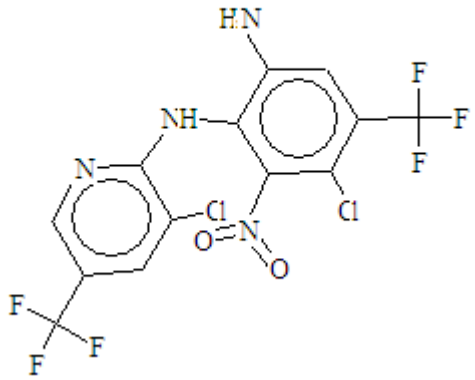
Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			RCOM, see Annex 2.2)	
		Repeated dose toxicity: - For oral repeated dose toxicity, in almost all studies the LOAEL lies between the cut off limits for classification as STOT-RE Cat 2; H373 or Xn; R48/22. Without a more extensive summary of the study results, including the quantity of effects and quality of effects ('histopathological changes' can be not relevant or very severe effects) it is not possible to conclude whether fluazinam should be classified for repeated dose toxicity. It should be noted that in one of the teratology studies in rabbits, focal hepatocytic necrosis was observed. - According to the CLP regulation, impurities may influence the classification of the substance. The fact that the changes in brain and spinal cord were not due to fluazinam itself, but rather to a manufactory impurity, called Impurity-5, is therefore no reason for not classifying fluazinam (assuming that impurity-5 is a regular impurity in fluazinam). - The results of the 2 year studies in rat and mice described under carcinogenicity should also be included in the discussion of repeated dose toxicity.	The studies mentioned have been peer reviewed by the experts of the PRAPeR 29 Meeting and the outcome is available in the EFSA conclusion.	The presentation of these studies, including the protocol and findings, should have been clarified and presented in more detail.
		Environmental Hazard Assessment General comments Due to the way the information is presented it is not easy to understand on first glance which information is considered most relevant for purposes of classification. The level of detail provided for each study is extensive and in our view not necessary for this type of dossier. According to the Guidance on the preparation of CLH dossiers, 'in the CLH report the relevant available information should be systematically evaluated in order to derive a classification and report should provide more concise and comprehensive overview of the scientific evidence.' Most pronounced are the detailed descriptions of the toxicity studies. We would recommend to present the outcome of all the reliable studies in a table and to give only a more detailed description of the key studies which drive the acute and chronic classification and M-factor	Will be amended in revised CLH report as part of the RCOM (Annex 2.2)	We agree that in some sections there is unnecessary information. Extensive information about the tests performed, such as very detailed descriptions of material and methods, is not relevant for classification purposes and therefore should not be included in
		It should be specified in the dossier if environmental fate properties and environmental hazard assessments were based on studies and summaries based on the Draft Assessment Report and its addenda. If this is the case, this source can be mentioned in a brief introduction in the headers of sections 4 and 6 and a statement that makes clear that only reliable studies accepted only for risk assessment are reported. This would avoid having to reference every study or summary (e.g. data source and study title) presented in the dossier. We also wonder what the relevant is of presenting the degradation studies performed in soil if these data are not	Will be amended in revised CLH report as part of the RCOM (Annex 2.2)	

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		considered in the overall C&L. The same accounts for the presented toxicity data for sediment dwelling organisms. In the absence of C&L criteria these data can not be used. More fundamental is the interpretation of the C&L criteria of Directive 67/548/EEC and Regulation EC 1272/2008 concerning the degradability. It is not correct to consider a substance not readily biodegradable according to Directive 67/548/EEC and rapidly degradable according to Regulation EC 1272/2008. Also according to Directive 67/548/EEC it would have been possible not to apply R53 based on the fact that the substance rapidly degrades in a water/sediment study. However, this can only be done when evidence is provided that the degradation products are not classifiable. Such evidence is not provided in this dossier. Therefore, based on the information in this dossier the substance can not be considered to be rapidly degradable. In the final conclusion nothing is mentioned on the bioaccumulation potential of the substances. This should be included as well. We would like to ask the rapporteur to adjust the report and conclusion accordingly and apply the appropriate M-factor both for the acute and chronic classification. Specific comments Page 69, Table 19 Summary of relevant information on degradation Water/Sediment; study: Please specify that DT50/DT90 values represent the arithmetic mean for two systems and radio labelled positions (phenyl and pyridine). Page 80, 5.1.3 Summary and discussion of degradation Please include a conclusion on degradability of the substance based the findings provided Page 83/84, 5.2 Environmental distribution Beside the fact that a number of studies regarding degradation (aerobic/anaerobic in soil), photolysis (soil) and field studies might be redundant, they are also placed incorrectly under environmental distribution. Page 88, 5.3.2 Summary and discussion of aquatic bioaccumulation No conclusions are provided whether the findings indicate that the substance fulfils or does not fulfil respective bioaccumulation criteria for classification and labelling purposes Page 101, 5.4.4 Other aquatic organisms (including sediment) Study not relevant for this type of dossier. Page 103, 5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)	Noted Will be amended in revised CLH report as part of the RCOM (Annex 2.2) Will be amended in revised CLH report (Annex 2.2) Will be amended in revised CLH report (Annex 2.2) Noted Will be amended in revised CLH report (Annex 2.2)	CLH report. This section is corrected in the revised CLH report as part of the RCOM. Fluazinam is considered not rapidly degradable. Corrected. Corrected. Corrected in rev. CLH report as part of the RCOM Agree with

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		The manner in which the information (e.g. justification and classification codes) is presented regarding the environment makes it difficult to follow the reasoning for the CLH proposal. We would like to you to consider restructuring this section. It is also useful to report justification for classification and labeling according to CLP regulation and DSD criteria separately. It is also our view that hazard class and the associated hazard statement for the CLP classification are used improperly. In Annex VI, Table 3.1 of the CLP regulation hazard classification is expressed using the following format: hazard class and category code(s) + hazard statement code(s). The classification according to CLP for fluazinam should be referred as Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). For consistency sake please make proper adjustments in the text and table. Pages 103/104, 5.5 Comparison with criteria for environmental hazards (this comment also applies to page 11, 2.2 Short summary of the scientific justification for the CLH proposal, regarding environment). Based on our comments mentioned above we would like to propose to change the classification of fluazinam based on the fact that the substances is not rapidly degradable in the absence of data showing that the degradation products are not classifiable. Consequently, an M-factor of 10 should also be applied to the chronic classification.	Noted Will be amended in revised CLH report (Annex 2.2) Will be amended in revised CLH report (Annex 2.2)	comment. Corrected in the revised CLH report as part of the RCOM Corrected in the revised CLH report as part of the RCOM
01/07/2011	Germany / Member State	Comment on behalf of the German CA: Acute toxicity: Generally DE agrees with the proposed classification for Fluazinam that is Xn; R20 and Acute Tox 4 – H332, respectively. Based on the data the inhalative LC40 of Fluazinam in male rats was 1.1 mg/l. Therefore, it can be concluded that the inhalative LC50 of Fluazinam is less than 5 mg/l. But it should be clarified that dust aerosol is the representative exposure and Fluazinam in combination with PEG 400 placing on the market can be excluded. Otherwise the original study from Tobeta, 1988 should be taken into consideration and a classification T; R23 and Acute Tox 2 – H330, respectively based on the lower LC50 values could be recommended. Furthermore the argumentation for limited validity of the Tobeta study relating to whole body exposure can not be followed. The results from the oral and dermal studies showed low respective no signs of acute toxicity in the tested doses. Specific target organ toxicity – Single exposure: DE questions the proposed classification for Fluazinam Xi; R37 and STOT SE 3 – H335, respectively. From the provided data it can not be concluded whether respiratory irritant effects also occur at lower concentrations. To avoid a dual classification resulting from the identical tests and concentrations the proposed classification should be reconsidered. Skin irritation:	The studies mentioned have been peer reviewed by the experts of the PRAPeR 29 Meeting and the outcome is available in the EFSA conclusion. Noted	Noted. Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Based on the given information, we disagree with the proposed classification Xi, R38 (DSD) and Skin irrit. 2 – H315 (CLP), respectively. After single exposure in rabbits, fluazinam causes indeed skin irritating reactions but these are reversible within a 14 day-observation period and do not fulfil the criteria for classification due to their low severity (Shults, 1992). In our opinion, it is inappropriate to refer to data from a dermal repeated dose toxicity study in rats (Cummins et al. 1985) where skin reactions were only evaluated at the end of a 21 days-exposure period. Also, scabs and ulcerations frequently occurred only after repeated dermal exposure to 1000 mg/kg bw. This dose level is about 4,5-times higher than that one used in the dermal acute rabbit study by Cummins et al. (~220 mg/kg bw) or usually applied in OECD TG404. Moreover, there is missing information about the severity of the observed acanthosis and dermatitis so that a comparison with the classification criteria cannot be conducted.	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 in the 21 day study.	Noted.
		Eye irritation: We agree with the proposed classification Xi, R41 (DSD) and Eye Dam.1 – H318 (CLP), respectively, due to the observed persistence of some effects in the eye throughout a 21 days-observation period after single exposure to fluazinam.	Noted	Noted.
		Respiratory tract irritation: Please see comment in section "STOT SE".	Noted	Noted.
		Corrosivity: Based on the given data, we agree with the conclusion that fluazinam is not corrosive.	Noted	Noted.
		Skin sensitisation: In our opinion, only the outcome of the Bühler-Test (Pritchard, 1986) is indicative of technical fluazinam having skin sensitising properties. The Magnusson & Kligman (M&K)-test (Cummins, 1984), however, does not allow for a clear conclusion because the observed skin reactions after challenge are very similar in control and treated animals in terms of incidence and severity of erythema development. It appears also doubtful that there are	Noted	Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		positive reactions after challenge with the vehicle paraffin oil only. In fact, only technical fluazinam is to be considered a moderate skin sensitiser whereas purified fluazinam does not fulfil the criteria for classification (see CLP-guidance chapter 3.4.2.3.4, page 271: significant skin sensitising effect if redness in ≥ 15 % of the test animals). We assume that technical fluazinam is used for manufacture of its corresponding pesticide products. Therefore, referring to fluazinam we agree with the proposed classification Xi, R43 (DSD) and Skin Sens.1 – H317 (CLP), respectively. Repeated dose toxicity: Based on the given information, the proposal seems justified not to classify fluazinam for repeated dose toxicity. We would like to mention, however, that the given data do actually not allow for an in-depth verification due to lacking nominal values. Labelling: Directive 67/548/EEC: Based on R63 (Possible risk of harm to the unborn child) S22 (Do not breathe dust) should be assigned. Regulation EC 1272/2008: No response precautionary statements (P3xx) are assigned. Please add appropriate Precautionary statements.	Noted Noted Noted (please refer to the comment of Finland also on page 4)	Noted. Noted. Noted.
04/07/2011	Spain / Member State	Degradation Fluazinam is rapidly degradable in water, in water/sediment simulation tests and in soil tests, but in water/sediment simulation tests, Fluazinam produces a stable metabolite identified as AMPA. This metabolite reaches concentrations of 22.5% and 15.8 % (mean measured) in Virginia water and Emperor Lake sediments respectively. In this study the AMPA DT50's were calculated for Emperor Lake system only showing values of 43.7 d and 24 d at 20ºC for the 14C pyridyl label and 14C phenyl label respectively, therefore AMPA doesn't fulfil the rapidly degradation criteria. Aquatic Toxicity We have done some AMPA QSAR's (EPISUITE v4.1) predictions (attached as AMPA2.zip), these predictions show a log Kow 3.99 and a predicted Fish 14d LC50 of 0.33 mg/L (n = 17 and r2 = 0.7564), we have chosen this toxicity prediction because the Fish 30d chronic toxicity (0.007 mg/L) model is not suitable (n = 17 and r2 =	We agree with the Spanish authorities. Please refer to CLH report, rev. 3, Point 5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4) Acute aquatic toxicity data for AMPA were	Corrected in version 3 of CLH report. Data for AMPA provided in the CLH report.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		0.32). Taking into account that the toxicity of some anilines are approximately 20 times greater than the toxicity predicted by the log Kow we recommended to keep the Chronic M factor of 10 until the AMPA toxicological information to be clarified. *ECHA comment: The document attached EPI Suite Results For CAS, (AMPA2.doc) is copied below:* EPI Suite Results For CAS  SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- EPI SUMMARY (v4.00) ---------- Physical Property Inputs: Log Kow (octanol-water): ------	available and will be amended in CLH report, rev. 3,	

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		Boiling Point (deg C) : ------ Melting Point (deg C) : ------ Vapor Pressure (mm Hg) : ------ Water Solubility (mg/L): ------ Henry LC (atm-m3/mole) : ------ KOWWIN Program (v1.68) Results: ========== Log Kow(version 1.68 estimate): 3.99 SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 -------+-----+----------+---------+-------- TYPE | NUM | LOGKOW FRAGMENT DESCRIPTION | COEFF | VALUE -------+-----+----------+---------+-------- Frag | 2 | C [aliphatic carbon - No H, not tert] | 0.9723 | 1.9446 Frag | 6 | -F [fluorine, aliphatic attach] |-0.0031 | -0.0186 Frag | 11 | Aromatic Carbon | 0.2940 | 3.2340 Frag | 1 | Aromatic Nitrogen |-0.7324 | -0.7324 Frag | 2 | -CL [chlorine, aromatic attach] | 0.6445 | 1.2890 Frag | 1 | -N [aliphatic N, one aromatic attach] |-0.9170 | -0.9170 Frag | 1 | -NO2 [nitro, aromatic attach] |-0.1823 | -0.1823 Frag | 1 | -N- [aliphatic N, two aromatic attach] |-0.4657 | -0.4657 Factor| 1 | Ring reaction -> -NO2 with -OH/amino/azo | 0.5777 | 0.5777 Factor| 1 | Ortho-Amino pyridine correction | 0.6421 | 0.6421 Factor| 1 | Pyridine ring (non-fused) correction |-0.1621 | -0.1621 Factor| 1 | Ortho rx: (-NO2/-N-) on diarylamine(o-Nar)|-0.9500 | -0.9500 Factor| 2 | Ortho rx: (to -N-) on diarylamine(o-Nar) |-0.2500 | -0.5000 Const | | Equation Constant | | 0.2290 -------+-----+----------+---------+-------- Log Kow = 3.9883			

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		MPBPVP (v1.43) Program Results: ========== Experimental Database Structure Match: no data SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- SUMMARY MPBVP v1.43 ---------- Boiling Point: 440.25 deg C (Adapted Stein and Brown Method) Melting Point: 349.84 deg C (Adapted Joback Method) Melting Point: 143.40 deg C (Gold and Ogle Method) Mean Melt Pt : 246.62 deg C (Joback; Gold,Ogle Methods) Selected MP: 184.69 deg C (Weighted Value) Vapor Pressure Estimations (25 deg C): (Using BP: 440.25 deg C (estimated)) (Using MP: 184.69 deg C (estimated)) VP: 1.63E-009 mm Hg (Antoine Method) : 2.18E-007 Pa (Antoine Method) VP: 1.84E-008 mm Hg (Modified Grain Method) : 2.46E-006 Pa (Modified Grain Method) VP: 4.72E-008 mm Hg (Mackay Method) : 6.29E-006 Pa (Mackay Method) Selected VP: 1.84E-008 mm Hg (Modified Grain Method) : 2.46E-006 Pa (Modified Grain Method) Subcooled liquid VP: 8.57E-007 mm Hg (25 deg C, Mod-Grain method) : 0.000114 Pa (25 deg C, Mod-Grain method) -------+-----+-------------------+----------+---------		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		TYPE | NUM | BOIL DESCRIPTION | COEFF | VALUE -------+-----+--------------------+----------+--------- Group | 2 | >C< | 4.50 | 9.00 Group | 6 | -F | 0.13 | 0.78 Group | 1 | >NH (nonring) | 45.28 | 45.28 Group | 1 | -NO2 (nitro) | 113.99 | 113.99 Group | 3 | CH (aromatic) | 28.53 | 85.59 Group | 8 | -C (aromatic) | 30.76 | 246.08 Group | 1 | -NH2 (to arom) | 86.63 | 86.63 Group | 1 | N (aromatic) | 39.88 | 39.88 Group | 2 | -Cl (to aromat) | 36.79 | 73.58 * | | Equation Constant | | 198.18 ==========+====================+==========+========= RESULT-uncorr| BOILING POINT in deg Kelvin | 898.99 RESULT- corr | BOILING POINT in deg Kelvin | 713.41 | BOILING POINT in deg C | 440.25 ---------- -------+-----+--------------------+----------+--------- TYPE | NUM | MELT DESCRIPTION | COEFF | VALUE -------+-----+--------------------+----------+--------- Group | 2 | >C< | 46.43 | 92.86 Group | 6 | -F | -15.78 | -94.68 Group | 1 | >NH (nonring) | 52.66 | 52.66 Group | 1 | -NO2 (nitro) | 127.24 | 127.24 Group | 3 | CH (aromatic) | 8.13 | 24.39 Group | 8 | -C (aromatic) | 37.02 | 296.16 Group | 1 | -NH2 (to arom) | 66.89 | 66.89 Group | 1 | N (aromatic) | 68.40 | 68.40 Group | 2 | -Cl (to aromat) | 13.55 | 27.10 * | | Equation Constant | | 122.50 ==========+====================+==========+========= RESULT | MELTING POINT in deg Kelvin | 783.52 RESULT-limit| MELTING POINT in deg Kelvin | 623.00 | MELTING POINT in deg C | 349.84		

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		---------- Water Sol from Kow (WSKOW v1.42) Results: ========== Water Sol: 0.726 mg/L SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- WSKOW v1.42 Results ---------- Log Kow (estimated) : 3.99 Log Kow (experimental): not available from database Log Kow used by Water solubility estimates: 3.99 Equation Used to Make Water Sol estimate: Log S (mol/L) = 0.796 - 0.854 log Kow - 0.00728 MW + Correction (used when Melting Point NOT available) Correction(s): Value ---------- ----- No Applicable Correction Factors Log Water Solubility (in moles/L) : -5.778 Water Solubility at 25 deg C (mg/L): 0.726 WATERNT Program (v1.01) Results: ========== Water Sol (v1.01 est): 0.29029 mg/L		

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		SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 -------+-----+----------+--------- TYPE | NUM | WATER SOLUBILITY FRAGMENT DESCRIPTION | COEFF | VALUE -------+-----+----------+--------- Frag | 2 | C [aliphatic carbon - No H, not tert] |-1.0516 | -2.1033 Frag | 6 | -F [fluorine, aliphatic attach] |-0.1580 | -0.9480 Frag | 3 | Aromatic Carbon (C-H type) |-0.3359 | -1.0076 Frag | 1 | Aromatic Nitrogen [max count of 1 allowed]| 1.9255 | 1.9255 Frag | 2 | -CL [chlorine, aromatic attach] |-0.4878 | -0.9756 Frag | 1 | -N [aliphatic N, one aromatic attach] | 1.2749 | 1.2749 Frag | 1 | -NO2 [nitro, aromatic attach] |-0.1915 | -0.1915 Frag | 8 | Aromatic Carbon (C-substituent type) |-0.5400 | -4.3196 Frag | 1 | -N- [aliphatic N, two aromatic attach] | 0.6988 | 0.6988 Factor| 1 | Ring reaction -> -Amino / -NO2 |-0.7786 | -0.7786 Const | | Equation Constant | | 0.2492 -------+-----+----------+--------- Log Water Sol (moles/L) at 25 dec C = -6.1758 Water Solubility (mg/L) at 25 dec C = 0.29029 ECOSAR Program (v1.00) Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA CAS Num: ChemID1: ChemID2: ChemID3: MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11		

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		Log Kow: 3.99 (KowWin estimate) Melt Pt: Wat Sol: 0.726 mg/L (WskowWin estimate) ECOSAR v1.00 Class(es) Found ---------- Anilines (Aromatic Amines) Halopyrdines Predicted ECOSAR Class Organism Duration End Pt mg/L (ppm) ========== ========== ======== ====== ========== Anilines (Aromatic Amines) : Fish 96-hr LC50 5.802 * Anilines (Aromatic Amines) : Fish 14-day LC50 0.331 Anilines (Aromatic Amines) : Daphnid 48-hr LC50 1.655 * Anilines (Aromatic Amines) : Green Algae 96-hr EC50 13.265 * Anilines (Aromatic Amines) : Fish ChV 0.007 Anilines (Aromatic Amines) : Daphnid ChV 0.014 Anilines (Aromatic Amines) : Green Algae ChV 7.027 * Halopyrdines : Fish 96-hr LC50 0.406 Halopyrdines : Daphnid 48-hr LC50 1.024 * Halopyrdines : Fish 32-day ChV 0.074 Halopyrdines : Daphnid ChV 0.029 ! ========== ========== ======== ====== ========== Neutral Organic SAR : Fish 96-hr LC50 6.963 * (Baseline Toxicity) : Daphnid 48-hr LC50 5.110 * : Green Algae 96-hr EC50 4.981 * : Fish ChV 0.633 : Daphnid ChV 0.671 : Green Algae ChV 2.592 * Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect.		

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		Note: ! = exclamation designates: The toxicity value was determined from a predicted SAR using established acute-to-chronic ratios and ECOSAR regression techniques which are documented in the supporting Technical Reference Manual. When possible, this toxicity value should be considered in a weight of evidence approach. Anilines (Aromatic Amines): ---------- For Fish Acute Toxicity Values: 2,3,5,6-Tetrachloroaniline is 19 times more toxic than predicted by this SAR. Tetrabromoaniline may be more toxic than predicted by this SAR as well. For Daphnid and Green Algae Toxicity Values: Tetrachloro- and tetrabrom-aniline may be 20 times toxic than predicted by this SAR. N-Substituted anilines are less toxic than predicted by these SARs; for these compounds, Neutral Organic SARs are used. ECOSAR v1.00 SAR Limitations: ---------- Maximum LogKow: >7.8 (Fish 96-hr LC50, Daphnid 48-h LC50) Maximum LogKow: >3.7 (Fish 14-day LC50) Maximum LogKow: >4 (Green Algae 96-hr EC50 and ChV) Maximum LogKow: >4.3 (Fish ChV) Maximum LogKow: >2.4 (Daphnid ChV) Maximum Mol Wt: 1000 Halopyrdines : ---------- For Fish and Daphnid Acute Toxicity Values: If the log Kow of the chemical is greater than 5.0, or if the compound is solid and the LC50 exceeds the water solubility by 10X, no effects at saturation are predicted for these endpoints. For Green Algae Acute Toxicity Values: If the log Kow of the chemical is		

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		greater than 6.4, or if the compound is solid and the EC50 exceeds the water solubility by 10X, no effects at saturation are predicted for these endpoints. For All Chronic Toxicity Values: If the log Kow of the chemical is greater than 8.0, or if the compound is solid and the ChV exceeds the water solubility by 10X, no effects at saturation are predicted for these endpoints. ECOSAR v1.00 SAR Limitations: ---------- Maximum LogKow: 5.0 (LC50) Maximum LogKow: 6.4 (EC50) Maximum LogKow: 8.0 (ChV) Maximum Mol Wt: 1000 Baseline Toxicity SAR Limitations: ---------- Maximum LogKow: 5.0 (Fish 96-hr LC50; Daphnid LC50) Maximum LogKow: 6.4 (Green Algae EC50) Maximum LogKow: 8.0 (ChV) Maximum Mol Wt: 1000 HENRYWIN (v3.20) Program Results: ========== Bond Est : 1.74E-012 atm-m3/mole (1.76E-007 Pa-m3/mole) Group Est: Incomplete SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- HENRYWIN v3.20 Results ---------- ----------+----------+---------+----------		

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		CLASS | BOND CONTRIBUTION DESCRIPTION | COMMENT | VALUE ----------+----------+---------+---------- HYDROGEN | 3 Hydrogen to Carbon (aromatic) Bonds | | -0.4629 HYDROGEN | 3 Hydrogen to Nitrogen Bonds | | 3.8505 FRAGMENT | 2 C-Car | | 0.3239 FRAGMENT | 6 C-F | | -2.5106 FRAGMENT | 10 Car-Car | | 2.6381 FRAGMENT | 2 Car-CL | | -0.0482 FRAGMENT | 1 Car-NO2 | | 2.2496 FRAGMENT | 2 Car-Nar | | 3.2564 FRAGMENT | 3 Car-N | | 2.1912 FACTOR | 1 ortho-NO2 to -N< | | -1.3400 ----------+----------+---------+---------- RESULT | BOND ESTIMATION METHOD for LWAPC VALUE | TOTAL | 10.148 ----------+----------+---------+---------- HENRYs LAW CONSTANT at 25 deg C = 1.74E-012 atm-m3/mole = 7.11E-011 unitless = 1.76E-007 Pa-m3/mole --------+----------+------------+-------- | GROUP CONTRIBUTION DESCRIPTION | COMMENT | VALUE --------+----------+------------+-------- | 1 NH2 (Car) | ESTIMATE | 4.00 | 2 Car (N)(Car)(Car) | ESTIMATE | -1.00 | 2 Car-H (Car)(Car) | | 0.22 | 1 Car-H (Car)(Nar) | | 0.11 | 2 Car (C)(Car)(Car) | | 1.40 | 2 Car (Car)(Car)(CL) | | 0.36 | 1 Car (Car)(Car)(NO2) | | 2.19 | 1 Nar (Car)(Car) | | 3.06 | MISSING Value for: C (F)(F)(F)(Car) | MISSING Value for: NH (Car)(Car) | MISSING Value for: Car (Nar)(Car)(N) | MISSING Value for: C (F)(F)(F)(Car) --------+----------+------------+--------		

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		RESULT | GROUP ESTIMATION METHOD for LOG GAMMA VALUE | INCOMPLETE | 10.34 --------+----------+-------- For Henry LC Comparison Purposes: Exper Database: none available User-Entered Henry LC: not entered Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]: HLC: 1.451E-008 atm-m3/mole (1.470E-003 Pa-m3/mole) VP: 1.84E-008 mm Hg (source: MPBPVP) WS: 0.726 mg/L (source: WSKOWWIN) Log Octanol-Air (KOAWIN v1.10) Results: ========== Log Koa: 14.138 SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- KOAWIN v1.10 Results ---------- Log Koa (octanol/air) estimate: 14.138 Koa (octanol/air) estimate: 1.374e+014 Using: Log Kow: 3.99 (KowWin est) HenryLC: 1.74e-012 atm-m3/mole (HenryWin est) Log Kaw: -10.148 (air/water part.coef.) LogKow : ---- (exp database) LogKow : 3.99 (KowWin estimate) Henry LC: --- atm-m3/mole(exp database) Henry LC: 1.74e-012 atm-m3/mole (HenryWin bond estimate)		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Log Koa (octanol/air) estimate: 14.138 (from KowWin/HenryWin) BIOWIN (v4.10) Program Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- BIOWIN v4.10 Results ---------- Biowin1 (Linear Model Prediction) : Does Not Biodegrade Fast Biowin2 (Non-Linear Model Prediction): Does Not Biodegrade Fast Biowin3 (Ultimate Biodegradation Timeframe): Recalcitrant Biowin4 (Primary Biodegradation Timeframe): Months Biowin5 (MITI Linear Model Prediction) : Does Not Biodegrade Fast Biowin6 (MITI Non-Linear Model Prediction): Does Not Biodegrade Fast Biowin7 (Anaerobic Model Prediction): Does Not Biodegrade Fast Ready Biodegradability Prediction: NO ------+-----+----------+---------+--------- TYPE | NUM | Biowin1 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -0.1824 | -0.3648 Frag | 1 | Aromatic nitro [-NO2] | -0.3050 | -0.3050 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.2338 | -0.4675 Frag | 1 | Pyridine ring | -0.1546 | -0.1546 Frag | 2 | Trifluoromethyl group [-CF3] | -0.5204 | -1.0408 MolWt| * | Molecular Weight Parameter | | -0.2071 Const| * | Equation Constant | | 0.7475 ==========+==========+=========+========= RESULT | Biowin1 (Linear Biodeg Probability) | | -1.7924 ==========+==========+=========+=========		

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		------+-----+----------+---------+--------- TYPE | NUM | Biowin2 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -2.0155 | -4.0310 Frag | 1 | Aromatic nitro [-NO2] | -2.5086 | -2.5086 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -1.9070 | -3.8140 Frag | 1 | Pyridine ring | -1.6381 | -1.6381 Frag | 2 | Trifluoromethyl group [-CF3] | -5.6696 |-11.3392 MolWt| * | Molecular Weight Parameter | | -6.1786 ============+==========+=========+========= RESULT | Biowin2 (Non-Linear Biodeg Probability) | | 0.0000 ============+==========+=========+========= A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast ------+-----+----------+---------+--------- TYPE | NUM | Biowin3 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -0.2066 | -0.4132 Frag | 1 | Aromatic nitro [-NO2] | -0.1696 | -0.1696 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.1349 | -0.2699 Frag | 1 | Pyridine ring | -0.2142 | -0.2142 Frag | 2 | Trifluoromethyl group [-CF3] | -0.5130 | -1.0259 MolWt| * | Molecular Weight Parameter | | -0.9615 Const| * | Equation Constant | | 3.1992 ============+==========+=========+========= RESULT | Biowin3 (Survey Model - Ultimate Biodeg) | | 0.1448 ============+==========+=========+========= ------+-----+----------+---------+--------- TYPE | NUM | Biowin4 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -0.1653 | -0.3307 Frag | 1 | Aromatic nitro [-NO2] | -0.1084 | -0.1084		

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		Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.1084 | -0.2168 Frag | 1 | Pyridine ring | -0.0187 | -0.0187 Frag | 2 | Trifluoromethyl group [-CF3] | -0.2744 | -0.5488 MolWt| * | Molecular Weight Parameter | | -0.6278 Const| * | Equation Constant | | 3.8477 ==========+==========+=========+========= RESULT | Biowin4 (Survey Model - Primary Biodeg) | | 1.9966 ==========+==========+=========+========= Result Classification: 5.00 -> hours 4.00 -> days 3.00 -> weeks (Primary & Ultimate) 2.00 -> months 1.00 -> longer ------+-----+----------+---------+--------- TYPE | NUM | Biowin5 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | 0.0062 | 0.0123 Frag | 1 | Aromatic nitro [-NO2] | -0.1876 | -0.1876 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.1577 | -0.3154 Frag | 1 | Pyridine ring | -0.0335 | -0.0335 Frag | 6 | Fluorine [-F] | 0.0174 | 0.1043 Frag | 3 | Aromatic-H | 0.0082 | 0.0247 MolWt| * | Molecular Weight Parameter | | -1.2945 Const| * | Equation Constant | | 0.7121 ==========+==========+=========+========= RESULT | Biowin5 (MITI Linear Biodeg Probability) | | -0.9775 ==========+==========+=========+========= ------+-----+----------+---------+--------- TYPE | NUM | Biowin6 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -0.2191 | -0.4383 Frag | 1 | Aromatic nitro [-NO2] | -2.4035 | -2.4035 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -1.2264 | -2.4527 Frag | 1 | Pyridine ring | -0.4599 | -0.4599 Frag | 6 | Fluorine [-F] | -3.9878 | -23.9271		

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		Frag | 3 | Aromatic-H | 0.1201 | 0.3604 MolWt| * | Molecular Weight Parameter | |-12.5612 ==========+==========+=========+========= RESULT |Biowin6 (MITI Non-Linear Biodeg Probability)| | 0.0000 ==========+==========+=========+========= A Probability Greater Than or Equal to 0.5 indicates --> Readily Degradable A Probability Less Than 0.5 indicates --> NOT Readily Degradable ------+-----+----------+---------+--------- TYPE | NUM | Biowin7 FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | -0.4023 | -0.8045 Frag | 1 | Aromatic nitro [-NO2] | -0.2141 | -0.2141 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.2778 | -0.5556 Frag | 1 | Pyridine ring | 0.6411 | 0.6411 Frag | 2 | Trifluoromethyl group [-CF3] | 0.0000 | 0.0000 Frag | 6 | Fluorine [-F] | 0.0000 | 0.0000 Frag | 3 | Aromatic-H | -0.0954 | -0.2863 Const| * | Equation Constant | | 0.8361 ==========+==========+=========+========= RESULT | Biowin7 (Anaerobic Linear Biodeg Prob) | | -0.3834 ==========+==========+=========+========= A Probability Greater Than or Equal to 0.5 indicates --> Biodegrades Fast A Probability Less Than 0.5 indicates --> Does NOT Biodegrade Fast Ready Biodegradability Prediction: (YES or NO) ---------- Criteria for the YES or NO prediction: If the Biowin3 (ultimate survey model) result is "weeks" or faster (i.e. "days", "days to weeks", or "weeks" AND the Biowin5 (MITI linear model) probability is >= 0.5, then the prediction is YES (readily biodegradable). If this condition is not satisfied, the prediction is NO (not readily biodegradable). This method		

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		is based on application of Bayesian analysis to ready biodegradation data (see Help). Biowin5 and 6 also predict ready biodegradability, but for degradation in the OECD301C test only; using data from the Chemicals Evaluation and Research Institute Japan (CERIJ) database. BioHCwin (v1.01) Program Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- BioHCwin v1.01 Results ---------- NO Estimate Possible ... Structure NOT a Hydrocarbon (Contains atoms other than C, H or S (-S-)) AEROWIN Program (v1.00) Results: ========== Sorption to aerosols (25 Dec C)[AEROWIN v1.00]: Vapor pressure (liquid/subcooled): 0.000114 Pa (8.57E-007 mm Hg) Log Koa (Koawin est): 14.138 Kp (particle/gas partition coef. (m3/ug)): Mackay model : 0.0263 Octanol/air (Koa) model: 33.7 Fraction sorbed to airborne particulates (phi): Junge-Pankow model : 0.487 Mackay model : 0.677 Octanol/air (Koa) model: 1 AOP Program (v1.92) Results: ==========		

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		SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- SUMMARY (AOP v1.92): HYDROXYL RADICALS (25 deg C) -------- Hydrogen Abstraction = 0.0000 E-12 cm3/molecule-sec Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec **Addition to Aromatic Rings = 0.7454 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 0.7454 E-12 cm3/molecule-sec HALF-LIFE = 21.524 Days (24-hr day; 0.5E6 OH/cm3) ..... ** Designates Estimation(s) Using ASSUMED Value(s) ---------- SUMMARY (AOP v1.91): OZONE REACTION (25 deg C) ---------- ****** NO OZONE REACTION ESTIMATION ****** (ONLY Olefins and Acetylenes are Estimated) Experimental Database: NO Structure Matches Fraction sorbed to airborne particulates (phi): 0.582 (Junge-Pankow, Mackay avg) 1 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation KOCWIN Program (v2.00) Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- KOCWIN v2.00 Results ----------		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Koc Estimate from MCI: ---------- First Order Molecular Connectivity Index ..... : 12.230 Non-Corrected Log Koc (0.5213 MCI + 0.60) ..... : 6.9753 Fragment Correction(s): 2 Nitrogen to non-fused aromatic ring ... : -1.0450 1 Nitro (-NO2) ..... : -0.4889 1 Pyridine ring ..... : -0.3080 Corrected Log Koc ..... : 5.1334 Estimated Koc: 1.36e+005 L/kg <========== Koc Estimate from Log Kow: ---------- Log Kow (Kowwin estimate) ..... : 3.99 Non-Corrected Log Koc (0.55313 logKow + 0.9251) .... : 3.1321 Fragment Correction(s): 2 Nitrogen to non-fused aromatic ring ... : -0.0432 1 Nitro (-NO2) ..... : 0.2191 1 Pyridine ring ..... : 0.1764 Corrected Log Koc ..... : 3.4843 Estimated Koc: 3050 L/kg <========== HYDROWIN Program (v2.00) Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- HYDROWIN v2.00 Results ---------- Hydrolyzable Function detected: Benzyl Halides Neutral hydrolysis half-lives of various Benzyl Halides (25 deg C)		

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		(Mabey and Mill, 1978; Laidler and Martin, 1969): Benzyl chloride: 15 hrs p-CH3 Benzyl chloride: 0.43 hrs p-CL Benzyl chloride: about 30 hrs p-NO2 Benzyl chloride: about 150-200 hrs Benzyl bromide: 1.32 hrs p-CH3 Benzyl bromide: 4.3 min Benzyl dichloride: 0.1 hrs Benzyl trichloride: 19 sec Ring substituents that may slow the hydrolysis rate: include CL, Br, I, NO2, cyano BCFBAF Program (v3.01) Results: ========== SMILES : C(F)(F)(F)c1c(CL)c(N(=O)(=O))c(Nc2c(CL)cc(C(F)(F)F)cn2)c(N)c1 CHEM : AMPA MOL FOR: C13 H6 CL2 F6 N4 O2 MOL WT : 435.11 ---------- BCFBAF v3.01 ---------- Summary Results: Log BCF (regression-based estimate): 2.30 (BCF = 199 L/kg wet-wt) Biotransformation Half-Life (days) : 0.3 (normalized to 10 g fish) Log BAF (Arnot-Gobas upper trophic): 2.06 (BAF = 114 L/kg wet-wt) Log Kow (experimental): not available from database Log Kow used by BCF estimates: 3.99 Equation Used to Make BCF estimate: Log BCF = 0.6598 log Kow - 0.333 + Correction Correction(s): Value No Applicable Correction Factors Estimated Log BCF = 2.298 (BCF = 198.8 L/kg wet-wt)		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		========== Whole Body Primary Biotransformation Rate Estimate for Fish: ========== ------+-----+----------+---------+--------- TYPE | NUM | LOG BIOTRANSFORMATION FRAGMENT DESCRIPTION | COEFF | VALUE ------+-----+----------+---------+--------- Frag | 2 | Aromatic chloride [-CL] | 0.3778 | 0.7557 Frag | 1 | Aromatic nitro [-NO2] | -0.0218 | -0.0218 Frag | 2 | Aromatic amine [-NH2 or -NH-] | -0.2890 | -0.5779 Frag | 1 | Pyridine ring | -0.9021 | -0.9021 Frag | 2 | Trifluoromethyl group [-CF3] | -0.1881 | -0.3763 Frag | 6 | Fluorine [-F] | 0.2759 | 1.6552 Frag | 3 | Aromatic-H | 0.2664 | 0.7991 Frag | 1 | Benzene | -0.4277 | -0.4277 L Kow| * | Log Kow = 3.99 (KowWin estimate) | 0.3073 | 1.2258 MolWt| * | Molecular Weight Parameter | | -1.1158 Const| * | Equation Constant | | -1.5058 ==========+==========+=========+========= RESULT | LOG Bio Half-Life (days) | | -0.5229 RESULT | Bio Half-Life (days) | | 0.3 NOTE | Bio Half-Life Normalized to 10 g fish at 15 deg C | ==========+==========+=========+========= Biotransformation Rate Constant: kM (Rate Constant): 2.311 /day (10 gram fish) kM (Rate Constant): 1.299 /day (100 gram fish) kM (Rate Constant): 0.7307 /day (1 kg fish) kM (Rate Constant): 0.4109 /day (10 kg fish) Arnot-Gobas BCF & BAF Methods (including biotransformation rate estimates): Estimated Log BCF (upper trophic) = 2.055 (BCF = 113.5 L/kg wet-wt) Estimated Log BAF (upper trophic) = 2.055 (BAF = 113.5 L/kg wet-wt) Estimated Log BCF (mid trophic) = 2.142 (BCF = 138.7 L/kg wet-wt) Estimated Log BAF (mid trophic) = 2.143 (BAF = 139 L/kg wet-wt) Estimated Log BCF (lower trophic) = 2.161 (BCF = 145 L/kg wet-wt)		

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		Estimated Log BAF (lower trophic) = 2.168 (BAF = 147.3 L/kg wet-wt) Arnot-Gobas BCF & BAF Methods (assuming a biotransformation rate of zero): Estimated Log BCF (upper trophic) = 3.003 (BCF = 1008 L/kg wet-wt) Estimated Log BAF (upper trophic) = 3.355 (BAF = 2265 L/kg wet-wt) Volatilization From Water ========== Chemical Name: AMPA Molecular Weight : 435.11 g/mole Water Solubility : ----- Vapor Pressure : ----- Henry's Law Constant: 1.74E-012 atm-m3/mole (estimated by Bond SAR Method) RIVER LAKE --- --- Water Depth (meters): 1 1 Wind Velocity (m/sec): 5 0.5 Current Velocity (m/sec): 1 0.05 HALF-LIFE (hours) : 7.019E+008 7.657E+009 HALF-LIFE (days) : 2.925E+007 3.19E+008 HALF-LIFE (years) : 8.007E+004 8.735E+005 STP Fugacity Model: Predicted Fate in a Wastewater Treatment Facility ========== (using 10000 hr Bio P,A,S) PROPERTIES OF: AMPA --- Molecular weight (g/mol) 435.11 Aqueous solubility (mg/l) 0		

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		Vapour pressure (Pa) 0 (atm) 0 (mm Hg) 0 Henry 's law constant (Atm-m3/mol) 1.74E-012 Air-water partition coefficient 7.11608E-011 Octanol-water partition coefficient (Kow) 9772.37 Log Kow 3.99 Biomass to water partition coefficient 1955.27 Temperature [deg C] 25 Biodeg rate constants (h^-1),half life in biomass (h) and in 2000 mg/L MLSS (h): -Primary tank 0.00 7963.57 10000.00 -Aeration tank 0.00 7963.57 10000.00 -Settling tank 0.00 7963.57 10000.00 STP Overall Chemical Mass Balance: --- g/h mol/h percent Influent 1.00E+001 2.3E-002 100.00 Primary sludge 1.70E+000 3.9E-003 17.03 Waste sludge 1.23E+000 2.8E-003 12.26 Primary volatilization 6.82E-010 1.6E-012 0.00 Settling volatilization 1.80E-009 4.1E-012 0.00 Aeration off gas 4.44E-009 1.0E-011 0.00 Primary biodegradation 6.19E-003 1.4E-005 0.06 Settling biodegradation 1.80E-003 4.1E-006 0.02 Aeration biodegradation 2.37E-002 5.4E-005 0.24 Final water effluent 7.04E+000 1.6E-002 70.39 Total removal 2.96E+000 6.8E-003 29.61 Total biodegradation 3.17E-002 7.3E-005 0.32		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Level III Fugacity Model (Full-Output): ========== Chem Name : AMPA Molecular Wt: 435.11 Henry's LC : 1.74e-012 atm-m3/mole (Henrywin program) Vapor Press : 1.84e-008 mm Hg (Mpbpwin program) Liquid VP : 6.99e-007 mm Hg (super-cooled) Melting Pt : 185 deg C (Mpbpwin program) Log Kow : 3.99 (Kowwin program) Soil Koc : 1.36e+005 (KOCWIN MCI method) Mass Amount Half-Life Emissions (percent) (hr) (kg/hr) Air 4.84e-006 516 1000 Water 1.92 4.32e+003 1000 Soil 70.2 8.64e+003 1000 Sediment 27.9 3.89e+004 0 Fugacity Reaction Advection Reaction Advection (atm) (kg/hr) (kg/hr) (percent) (percent) Air 4e-016 0.00219 0.0163 7.29e-005 0.000543 Water 1.07e-017 104 646 3.45 21.5 Soil 1.61e-018 1.9e+003 0 63.2 0 Sediment 2.87e-017 167 188 5.57 6.25 Persistence Time: 1.12e+004 hr Reaction Time: 1.55e+004 hr Advection Time: 4.04e+004 hr Percent Reacted: 72.2 Percent Advected: 27.8 Half-Lives (hr), (based upon Biowin (Ultimate) and Aopwin): Air: 516.5 Water: 4320		

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Soil: 8640 Sediment: 3.888e+004 Biowin estimate: 0.145 (recalcitrant) Advection Times (hr): Air: 100 Water: 1000 Sediment: 5e+004		
01/07/2011	Spain / Member State	p. 29 Summary and discussion on acute toxicity Acute inhalation toxicity There are two studies for acute inhalation toxicity of fluazinam. The first one (Tobeta Y.; 1988, DAR) was carried out with a dilution in polyethylene glycol 400 and 4 hours exposure period (whole body exposure). The results of this study were LC50 0.463 mg/l for males and 0.476 mg/l for females. The second study in rats (Kirkpatrick D.;2006) was performed with tecnical fluazinam administered as a dust aerosol (nose only exposure). The result of this study was: CL50 >1.1 mg/l. The Spanish CA supports the Austria proposal for classification of fluazinam as Acute Tox. 4 (inhalation) H332: Harmful if inhaled according to Regulation EC 1272/2008 and as Xn; R20 Harmful by inhalation according to Directive 67/548/EC, based on the result obtained in the second study because the administration route is considered more representative of the potencial exposure. p. 30 Summary and discussion of Specific target organ toxicity –single exposure In the CLH report it was considered that fluazinam should be classified as Xi; R37 Irritating to respiratory system according to Directive 67/548/EC and as STOT SE cat. 3; H335 May cause respiratory irritation, according to Regulation EC 1272/2008. This classification is based on the clinical signs observed in the first acute inhalation study (Tobeta, 1988): hyperaemia and haemorrhage in the lungs, pulmonary emphysema and white foam in the trachea. Deaths were considered mostly to respiratory failure. However, in the second acute inhalation study (Kirkpatrick D.;2006) the only macroscopic finding noted was dark red discoloration of the lungs in 1 male that died. Spanish CA doesn´t support a classification regarding Specific target organ toxicity -single exposure based on the effects that appear in the first study. The administration route used in this study is not representative of the potencial exposure, so that, the effects observed could be due to the vehicle used. Besides, these effects were not observed in the second study where the administration of tecnical fluazinam, as a dust aerosol, is more representative of the potencial exposure. p. 32 Summary and discussion of skin irritation The Spanish CA supports the proposed classification of fluazinam as Xi; R38 (Irritating to skin) according to	Noted Noted Noted	Noted. Noted. Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		Directive 67/548/EC and as Skin Irrit. 2 (H315: Causes skin irritation) according to Regulation EC 1272/2008. The mean values of erythema and edema observed in the skin irritation study in rabbits (Shults, SK, 1992) are not sufficient for classification. However, this classification is based on macroscopic and microscopic changes (acanthosis and dermatitis at 10 mg/kg bw, scabs and ulceration at 100 mg/kg bw) observed in the skin in the dermal repeat dose study (3 weeks) in rats (Cummins, H.A; 1985). p. 34 Summary and discussion of eye irritation The Spanish CA supports the proposed classification of fluazinam as Xi; R41 (Risk of serious damage to eyes) according to Directive 67/548/EC and as Eye Irrit. 1 (H318: Causes serious eye damage) according to Regulation EC 1272/2008. This classification is based on the fact that some effects (iris lesions, vascularisation and opacity corneal) persisted till termination (day 21) of the eye irritation study in rabbits (Shults, S.K., 1992). p. 38 Summary and discussion of skin sensitisation The Spanish CA supports the proposed classification of Fluazinam as skin sensitizer; R43 (May cause sensitisation by skin contact) according to Directive 67/548/EC and as Skin Sens. 1B (H317: May cause an allergic skin reaction) according to Regulation EC 1272/2008. This classification is based on the positive response obtained in more than 30% of the guinea pigs in the dermal maximization study Buehler-test (Pritchard, V.A.; 1986)	Noted Noted	Noted. Noted.
n31/05/2011	Denmark / Cheminova A/S / Company-Manufacturer	p. 30: Regarding classification as skin irritant category 2 (H315) The classification as skin irritant is based on the study by Shults, S. K. (1992); report no. 5016-91-0281-TX-001. In this study it was found that fluazinam is slightly irritating (index 0.9 according to Draize). This should not give rise to a CLP classification. The proposed classification is based on the fact that irritation was observed after repeated exposure in another study. It is highly unusual and does not confirm the classification criteria to classify in this way, especially since another safety phrase is available to express this hazard (EUH 066). We have in our possession a skin irritation study on fluazinam (Sanders, A. (2006), a GLP study according to OECD 404, project no. 0545/0409). This study confirms the finding that fluazinam is a mild skin irritant. (index 0.7 according to the Draize). We are of course willing to submit the study if required.	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 in the 21 day study.	Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		p. 31: Regarding classification for eye damage category 1 (H318) We have in our possession an eye irritation study on fluazinam (Sanders, A. (2006), a GLP study according to OECD 405, project no. 0545/0410). In this study it was found that fluazinam was a moderate eye irritant. [CLP classification: Eye irritation Category 2 (H319)] We are of course willing to submit the study if required.	Noted	Noted.
04/07/2011	United Kingdom / UK Competent Authority	Acute Toxicity P26, Table 11. For clarity, please state in the table that the results indicated are the LD50/LC50. P29. In sections 4.2.3 to 4.2.5, it is not clear why the proposed classification has been made based on the second inhalation study. In this study, only one concentration (1.1 mg/ml) was tested. Therefore, the only conclusion that can be conclusively drawn is that the LC50 is >1.1mg/l not that the substance warrants classification. The results of the first study (LC50 = 0.463 mg/l in males), support a classification of Acute Tox 2 (H330) under CLP and R23 under Dir 67/548/EEC for inhalation exposure. Please provide further clarification for the proposed classification based on the results of the 2 studies.	Will be amended in Rev. 3 of the CLH Report. In the repeat inhalation toxicity study (*Kirkpatrick, 2006*) the endpoint obtained is LC_{50} > 1.1 mg/l air and demonstrates that the first study provided in an unrealistic exposure. The improved toxicological result can be attributed to the vehicle effect and the improved study design to exclude other than inhalation exposure. During the EU evaluation this rationale was	Done. Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
			accepted, at the meeting PRAPeR 29 (0519 – 20 07.2007) experts have agreed that the repeat study was more reliable to base the classification for acute toxicity by inhalation upon. The experts also concluded that the value LC50 >1.1 mg/L was not the highest dose technically achievable and according to this, the proposed classification was Xn, R20 Harmful by inhalation corresponding to Cat. 4, H332 under the CLP regulation. This was confirmed in the Peer Review report prepared by EFSA	
		STOT SE P30 section 4.3.3. (see also p35). According to the regulation, STOT is defined as non lethal target organ toxicity	Noted	Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		arising from a single exposure to a substance or mixture. Since the deaths in the acute inhalation studies appear to have been attributable to effects in the lungs, we do not believe an additional classification for STOT is required as these effects are already covered by the acute inhalation classification. Irritation Skin P32. Section 4.4.1.4. The rabbit data presented do not meet the criteria for classification under CLP or Dir 67/548/EEC. Instead, classification has been proposed on the basis of a repeated dose study in rats. Whilst it is possible to classify this end point using data from repeated dose studies under Directive 67/548/EEC, there is insufficient information presented in the dossier to allow the reader to make an independent judgement as to whether classification is appropriate. It would be helpful if information on the number of animals affected, the effects seen and the time scales in which they occurred were included. Also, it would be useful to discuss the results in line with the proposed positive results seen in the skin sensitisation studies. Eye p. 32, section 4.4.2 We agree with the classification proposal due to the irreversibility of effects. Respiratory Irritation P.35. We consider classification for respiratory irritation should be based upon effects observed in the nasal and upper respiratory tract regions rather than the lung. As such we do not support classification with R37 or STOT-SE 3 (see earlier comments on STOT-SE). Skin Sensitisation P36 Section 4.6.1.1. The criterion for a positive response in a guinea pig maximisation test is redness in 30 % of test animals. In a study using 20 animals, a positive response must be observed in at least six animals to warrant classification. The study summary is not clear, but If we have understood it, in the Magnusson and Kligman study, erythema was identified in six test animals 24 h after challenge and only in 2 animals at 48 hours.	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 in the 21 day study. Noted Noted Noted	Noted. Noted. Noted.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		However, erythema was also observed in 5 control animals at 24 hours and 1 control animal at 48 hours. This suggests that the erythema was more likely due to irritation then sensitisation. As such, we questions whether the results of this study support classification for skin sensitisation. P37: Insufficient information has been provided in the summary of the Bhueler study to conclude whether the response was a true sensitisation response or due to irritation.	Noted	Noted.
		Repeat Dose Toxicity P40, section 4.7.1.1.From the information provided it is not possible to determine which effects were observed below the cut-off for classification or the incidence or severity of these effects. P.42 Vacuolation of the white matter in the brain has been attributed to 'impurity 5'. Confidential information on the impurities is only provided in DAR – Vol 4 which is attached to the ICULID. It is therefore very difficult to assess the relevance of this 'impurity 5' to the classification of fluazinam. Please provide evidence supporting this conclusion and clearly explain the basis upon which no classification for fluazinam is warranted.	The studies mentioned have been peer reviewed by the experts of the PRAPeR 29 Meeting and the outcome is available in the EFSA conclusion.	Noted.
		Environmental Hazard Assessment We agree with the environmental classification and labelling proposal.		
		The environmental proposal is based on acute ecotoxicity data. The 2nd Adaption to Technical Progress to Regulation EC 1272/2008 (Commission Regulation No 286/2011) entered into force on 19 April 2011 and now includes chronic criteria for environmental classification. We feel the proposal should reflect the new criteria although note it would not change the proposed environmental classification or M factor.	Noted	Chronic data are included in the CLH report.
		We note the substance was reviewed under Directive 91/414/EEC and the assessment indicates ecotoxicity data for some aquatic degradants are available. As the substance rapidly dissipates (<16 days), we feel the proposal would benefit from including this information to confirm that the degradants are not more ecotoxic than the parent and proposed M factor is appropriate.	Acute aquatic toxicity data for AMPA were available and will be amended in CLH report, rev. 3 Chronic M factor of 10 was proposed	Ok.

Date	Country/ Organisation/ MSCA	Comment	Dossier submitter's response to comment	RAC's response to comment
		The dossier indicates the substance has a pKa of 7.4, which suggests the degree of ionisation may vary across an environmentally relevant pH range. Based on this we feel the dossier should consider the significance of the measured pH for the results observed in the ecotoxicity studies.	All relevant ecotoxicity studies were well performed according to guidelines within recommended pH ranges.	Ok.

ATTACHMENTS RECEIVED:

GENERAL COMMENTS

Makhteshim Chemical Works, *Summary of acute toxicity of Fluazinam technical*, Table 1. (**table 1.docx**) – Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Griffiths D R, 2009, *MCW 465 tech: Acute Inhalation Toxicity (Nose Only) Study in the Rat*, Project Number 0306/0391, Sponsor Number R – 24975. (**R-24975.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Acute Dermal Toxicity Study of MCW 465 In Rats*, Laboratory of Pharmacology and Toxicology, Sponsor No R-20270 (**R-20270.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Acute Oral Toxicity Study of MCW 465 in Rats*, Laboratory of Pharmacology and Toxicology, Sponsor No R-20269, Germany (**R-20269.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Acute Dermal Irritation/Corrosion Test (Patch Test) of MCW 465 in Rabbits*, Laboratory of Pharmacology and Toxicology, Sponsor No R-20272, Germany (**R-20272.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Acute Eye Irritation/Corrosion Test of MCW 465 in Rabbits*, Laboratory of Pharmacology and Toxicology, Sponsor No R-20273, Germany (**R-20273.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Examination of MCW 465 in the Skin Sensitisation Test in Guinea Pigs according to Magnusson and Kligman (Maximisation Test)*, Laboratory of Pharmacology and Toxicology, Sponsor No R-20274, Germany (**R-20274.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

TOXICITY TO REPRODUCTION

Letter from ISK Biosciences Europe N.V 04/07/2011, "*CLH report – Proposal for classification and labeling of the active substance fluazinam*", (**IBE comment fluazinam classification proposal.pdf)** – Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Ishihara Sangyo Kaisha, Ltd., 2006, Final Report – *A Prenatal Developmental Toxicity Study of Technical Fluazinam in Rats*, WIL-282006, Japan. (**Reference 2.pdf) –** Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Sato M., Wada K. et al. 2000, Influence of corn oil and Diet on Reproduction and the Kidney in Female Sprague-Dawley Rats, *Toxicological Sciences* 56, 156 - 164, Japan. (**Reference 4.pdf**) - Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Padmanabhan R., Ahmed I., 1997, Retinoic Acid-Induced Asymmetric Craniofacial Growth and Cleft Palate in the To Mouse Fetus, *Reproductive Toxicology*, Vol. 11, No.6, pp.843-860, United Arab Emirates. (**Reference 5.pdf)** - Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Beurskens et al, 2010, Retinol Status of Newborn Infants is Associated With Congenital Diaphragmatic Hernia, *Pediatrics* Volume 126, Number 4, p. 712-720, Canada. (**Reference 6.pdf**) - Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Kling D.E., Schnitzer J.J, 2007, Vitamin A Deficiency (VAD), Teratogenic, and Surgical Models of Congenital Diaphragmatic Hernia (CDH), *American Journal of Medical Genetics* Part C (Seminars in Medical Genetics) 145C; 139-157. (**Reference 7.pdf) -** Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Singh J.D, *Teratogenicity of palm oil in albino rats,* Nigeria. (**Reference 8.pdf) -** Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

Bottomley A.M., Willoughby C.R., 2006, *Fluazinam (B-1216) Overview of Embryo-fetal studies in the CD rat*, ISK0277/060106 (**Reference 9.pdf) -** Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

RESPIRATORY SENSITISATION

Letter from ISK Biosciences Europe N.V 04/07/2011, "*CLH report – Proposal for classification and labeling of the active substance fluazinam*", (**IBE comment fluazinam classification proposal.pdf)** – Submitted by Belgium / ISK Biosciences Europe N.V. / Company-Manufacturer

OTHER HAZARDZ AND ENDPOINTS

EPI Suite Results For CAS, (**AMPA2.doc)** – Submitted by Spain / Manuel Carbo / Member State

Makhteshim Chemical Works, *Summary of acute toxicity of Fluazinam technical,* Table 1. (**table 1.docx**) – Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Griffiths D R, 2009, *MCW 465 tech: Acute Inhalation Toxicity (Nose Only) Study in the Rat*, Project Number 0306/0391, Sponsor Number R – 24975. (**R-24975.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

Leuschner P, 2006, *Acute Dermal Toxicity Study of MCW 465 in Rats*, Laboratory of Pharmacology and Toxicology, Sponsor No R-2027, Gernamy (**R-20270.pdf**) - Submitted by Switzerland / Christian Strupp / Makhteshim Agan Holding B.V.

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Annex 2.2: The report below is a revision of the original CLH report that was performed by the dossier as part of the response to comments received under public consultation.

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 :

Austrian Agency for Health and Food Safety

Institute for Plant Protection Products Evaluation and Authorisation

Spargelfeldstraße 191, 1220 Vienna

Austria

Version number: 3 **Date:** **23.08.2011**

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8 ANNEXES 170

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Skin Irrit. 2 - H315 Skin Sens.1A - H317 Eye Dam. 1 - H318 Acute Tox. 4 - H332 STOT SE 3 - H335 Repr. 2 - H361 Aquatic Acute 1 - H400 (M-factor 10) Aquatic Chronic 1 - H410	Xn, R20 Xi, R37/38 Xi, R41 Xi, R43 Repr. Cat. 3, R63 N; R50/53

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

SCLs

Classification	Concentration [Cn in %]
N, R50/53	Cn ≥ 25
N, R50/53	2.5 ≤ Cn < 25
N, R51/53	0.25 ≤ Cn < 2.5
R52/53	<0.25 Cn

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: 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				Data lacking
3.4.	Skin sensitisation	Cat. 1A, H317		Cat. 1A, 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 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	Classification	Concentration [Cn in %]		
		N, R50/53	Cn ≥ 25		
		N, R50/53	2,5 ≤ Cn < 25		
		N, R51/53	025 ≤ Cn < 2,5		
		R52/53	<0.25 Cn		

[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. The justification for flammability, explosive and corrosive properties is given below:

Flammability: The test substance could not be ignited by a flame. After removal of the ignition source, no more sparks were observed. According to EEC/A10 no further testing is required. Therefore, technical fluazinam is not considered fpr classification and labelling with respect to flammability under the test condition mentioned.

Explosive properties: technical fluazinam has been tested for its explosive properties according to 3 test systems:
1. Thermal sensitivity test: no explosion after 5 minutes (nozzle diameter: 2.0 mm)
2. Shock test: no explosion occurred within 6 tests using a mass of 10 kg from a height of 0.4 m
3. Friction test: no explosion occurred within 6 tests using a 360 N loading
Based on the results of the 3 test systems made available, no classification and labelling with respect to explosive properties is regarded applicable.

Corrosive property to metals: No test was provided by the manufacturer. Since the test substance is solid (melting point > 55°C), no classification is considered necessary.

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 M-Factor 10 (CLP **aquatic environment hazards acute category 1** very toxic to aquatic organisms) and H410 M-Factor 10 (CLP **aquatic environment hazards chronic category 1** Very toxic to aquatic life with long lasting effects**)** are proposed.

R50 and H400 follows from the acute 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 and on the observed potential for bioaccumulation

H410 is based on the non rapid degradability 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).

Annex 2.2. Fluazinam Resubmitted CLH report

Part B.

SCIENTIFIC EVALUATION OF THE DATA

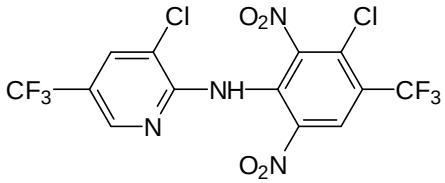
1 IDENTITY OF THE SUBSTANCE

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)-ααα- 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></table>ε above 290 nm in alkaline solution > 10</td><td>The UV spectra show in neutral and acidic media additional absorbance at approx. 340 nm, which is not reported. Data requirement see volume 1 level 4.</td><td>van Rijsbergen, L.M. (2002) (Document 341167)</td></tr>
<tr><td>US EPA Product Properties Tst Guidelines OPPTS 830.7050
GLP</td><td>Purified product (purity: 99.7% w/w) c = 4.66 x 10^{-5} mol/L
<table><tr><th>pH</th><th>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></table>ε above 290 nm in neutral and alkaline solution > 10</td><td>The UV spectrum shows in acidic medium additional absorbance at approx. 350 nm, which is not reported.</td><td>Gallacher, A.C. (1997) (Document 4039-97-0017-AS-001)</td></tr>
</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 ion 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: LD_{50} m/f > 5000 mg/kg bw	Decreased motor activity	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat: LD_{50} m/f > 5000 mg/kg bw	Decreased motor activity, hunched posture, piloerection, ataxia	*Cummins, 1988*
Acute oral toxicity	Sprague Dawley Rat: LD_{50} m: 4500 mg/ kg bw f: 4100 mg/ kg bw	Hunched posture, piloerection, lethargy, diarrhoea	*Liggett, 1988*
Acute oral toxicity	CD® (Crl: CD®) Rat LD_{50} m/f > 2000 mg/ kg bw	No signs of toxicity	Chevalier, 2006

Acute dermal toxicity	Sprague Dawley Rat: LD_{50} m/f > 2000 mg/kg bw	No reaction to treatment	*Cummins, 1988*
Acute dermal toxicity	CD® (Crl: CD®) Rat LD_{50} m/f > 2000 mg/ kg bw	No signs of toxicity	Chevalier, 2006
Acute inhalation toxicity	Sprague Dawley Rat: LC_{50} m: 0.463mg/l air LC_{50} 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: LC_{50} m/f > 1.1 mg/l air (4h, nose only exposure)	Dark red discoloration of the lungs.	*Kirkpatrick, 2006*
Acute inhalation toxicity	HsdRCCHan™: WIST Rat; mean achieved atmosphere concentration > 4.82 mg/L	Haemorrhagic or pale lungs, red discoloration, dark patches.	Griffiths, 2009

Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, owns data with regards to classification of Fluazinam (=MCW 465). The data were generated in European Contract Research Institutes in 2006/2009 under GLP.

4.2.1 Non-human information

4.2.1.1 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.

The acute oral (Chevalier, 2006) toxicity study (Chevalier, 2006) of Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, confirm that no classification for these endpoint is required:
Acute toxicity: oral

Report:	KIIIA1 7.11/01, Chevalier, F., 2006
Annex II data point:	IIA 5.2.1
Title:	Acute oral toxicity study of MCW 465 in rats
Testing facility:	LPT, Hamburg, Germany
Document No:	19774/06, Sponsor report no. R-20269
Guidelines:	OECD 423 (2001), EC method B.1 tris (2004/73/EC) Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary

Acute oral toxicity of MCW 465 (99 % fluazinam) was investigated according to the Acute Toxic Class (ATC) method. Three female rats received a single oral dose of 2000 mg/kg bw of the test item suspended in vehicle by gavage. None of the animals died. In a next step three male rats received the same single oral dose. No mortality did occur. The animals were observed for deaths or clinical signs for 14 days. Body weights were determined prior to dosing and weekly thereafter. All animals were subjected to gross necropsy.
No mortality occurred and no signs of systemic toxicity were noted in the animals dosed at 2000 mg/kg bw. No effects on body weight were observed and no abnormalities were noted upon necropsy.
Therefore, the acute oral median lethal dose (LD_{50}) of fluazinam was established above 2000 mg/kg bw. Thus, no classification is required according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations, (LD_{50} oral, rat: LD_{50} > 2000 mg/kg bw).

4.2.1.2 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

Sex	Actual Concentration	Day								
		0	1	2	3	4	5	6	7-14	Fin. Mort
	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
	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.

In the acute inhalation toxicity study (Griffiths, 2009) of Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, no mortality was observed up to the limit concentration:

Acute inhalation toxicity (nose only) study in the rat

Report:	KIIIA1 7.11/03, Griffiths, D.R., 2009
Annex II data point:	IIA 5.2.3
Title:	MCW 465 tech: Acute inhalation toxicity (nose only) study in the rat
Testing facility:	Harlan Laboratories Ltd., Shardlow, UK
Document No:	0306/0391, Sponsor no.: R-24975
Guidelines:	OECD Guideline for testing of chemicals 403 (1981), EU method B.2 (92/69/EEC) and corrected in Dir 93/21/EEC Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary

The acute inhalation toxicity of MCW 465 (fluazinam) technical was tested according to the guideline OECD 403. Groups of five male and five female rats were exposed to a dust atmosphere of the ground test material at a mean achieved test atmosphere of 2.08, 3.28 and 4.82 mg/L (gravimetrically determined) for a period of 4 hours under nose-only conditions. The particle size distribution was determined three times during the exposure period using a cascade impactor (six impactor stages).

The animals were observed for clinical signs at hourly intervals during exposure, immediately on removal from the restraining tubes at the end of exposure, one hour after termination of exposure and subsequently once daily for fourteen days. Body weights were determined prior to treatment and weekly thereafter. All animals included those that died or were humanely killed during the study, were subjected to gross pathology with a detailed macroscopic examination for evident lesion, signs of irritancy or local toxicity.

Deaths occurred in the low dosed (1 female) and in the intermediate (2 females) dosed group. No deaths occurred in the high dosed group of ten rats exposed for 4 h to the mean achieved atmosphere concentration of 4.82 mg/L air (nose only). Apart from common abnormalities hunched posture, pilo-erection, wet fur), there were frequent instances of decreased respiratory rate and laboured respiration observed. Occasional occurrences of noisy respiration and red/brown staining around the snout were also noted. All animals appeared normal and no further clinical signs were noted throughout the recovery period on day 10. With the exception of two instances of dark patches on the lungs of a male and female animal from the low dose group no macroscopic abnormalities were detected amongst animals that survived until day 14 at necropsy.

The highest achieved concentration did not cause acute inhalation toxicity up to a concentration practically equivalent to the limit concentration of 5 mg/L (mean maximal achieved atmosphere concentration was 4.82 mg/L).

4.2.1.3 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.

The acute dermal toxicity study (Chevalier, 2006) of Makhteshim Chemical Works Ltd., Beer-Sheva, Israel confirm that no classification for these endpoint is required:

Acute percutaneous toxicity in rats

Report:	KIIIA1 7.11/02, Chevalier, F., 2006
Annex II data point:	IIA 5.2.2
Title:	Acute dermal toxicity study of MCW 465 in rats
Testing facility:	LPT, Hamburg, Germany
Document No:	19775/06, Sponsor report no. R-20270
Guidelines:	OECD 402 (1987), EC method B.3 (92/69/EEC) Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary

MCW 465 (99 % fluazinam) suspended in sesame oil was applied to the shaved intact dorsal skin (5 × 6 cm², approx. 10 % of body surface) of 5 male and 5 female rats at a dose level of 2000 mg/kg and covered with an semi-occlusive dressing for 24 hours. At the end of the exposure period no residual test item had to be removed.

The animals were observed regularly for deaths and clinical signs of toxicity and skin reactions. Body weights were determined prior to application and weekly thereafter. Surviving animals were sacrificed on day 14 after application and all animals were subjected to gross pathology.

No signs of systemic toxicity and no deaths were noted in the animals dosed at 2000 mg/kg bw. No effects on body weight were observed during the test period and no abnormalities were found upon necropsy.

Therefore, the acute dermal median lethal dose (LD_{50}) of fluazinam was established above the limit dose of 2000 mg/kg bw. Thus, according to the classification criteria of Council Directive 67/548/EEC and subsequent regulations, no classification is required for fluazinam (LD_{50} dermal rat or rabbit > 2000 mg/kg bw).

4.2.1.4 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.

Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, owns data with regards to classification of Fluazinam (=MCW 465). The data were generated in European Contract Research Institutes in 2006/2009 under GLP.
The acute oral (Chevalier, 2006) and dermal toxicity studies (Chevalier, 2006) confirm that no classification for these endpoints is required. In the acute inhalation toxicity study (Griffiths, 2009) the highest achieved concentration did not cause acute inhalation toxicity up to a concentration practically equivalent to the limit concentration of 5 mg/L.
The differences in toxicological properties of technical Fluazinam evaluated for Annex I inclusion and Makhteshim's Fluazinam technical are potentially a consequence of 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 , but absent from Makhteshim's technical material (see EFSA Scientific review 2008, 137).

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).

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 3 - H335 Specific target organ toxicity –single exposure 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 STOT SE 3 - H335 Specific target organ toxicity –single exposure, signal word "Warning".

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*
Dermal irritation study	Rabbit (Himalayan):	Non-irritating	*Leuschner, 2006*

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.

In the dermal irritation study (Leuschner, 2006) of Makhteshim Chemical Works Ltd., Beer-Sheva, Israel, no erythema and no oedema were observed in any rabbit:

Skin irritation

Report:	KIIIA1 7.11/04, Leuschner, J., 2006
Annex II data point:	IIA 5.2.4
Title:	Acute dermal irritation/corrosion test (patch test) of MCW 465 in rabbits
Testing facility:	LPT, Hamburg, Germany
Document No:	19777/06, Sponsor report no. R-20272
Guidelines:	OECD 404 (2002), EC method B.4 (2004/73/EC) Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary
Three male Himalayan rabbits were exposed to 0.5 g of the moistened (with aqua ad iniectabilia) test substance MCW 465 (99 % fluazinam) applied to the shaved intact skin and covered with a semi-occlusive dressing for 4 hours. Approx. 1 hour, 24, 48 and 72 hours following patch removal, the test sites were examined for primary irritation and scored according to OECD and EU guidelines.
No erythema and no oedema were observed in any rabbit.

Individual skin irritation scores in albino rabbits with fluazinam technical

Rabbit No.	Time after treatment	Erythema	Oedema
M1	1 hour	0	0
	24 hours 48 hours 72 hours	0 0 0	0 0 0
M2	1 hour	0	0
	24 hours 48 hours 72 hours	0 0 0	0 0 0
M3	1 hour	0	0
	24 hours 48 hours 72 hours	0 0 0	0 0 0

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 in the study of *Shults, 1992*.

No erythema and no oedema were observed in any rabbit 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 Fluazinam technical are potentially a consequence of 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 , but absent from Makhteshim's technical material (see EFSA Scientific review 2008, 137).
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).
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

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 Skin Irrit. 2 - H315 and labeled with signal word "Warning", 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 Skin Irrit. 2 - H315 and labeled with signal word "Warning".

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

	Mean 24 – 72 h	1	0.3	2.6	2
202732	1 hour	0	0	2	2
	24 hours 48 hours 72 hours	1 1 1	0 0 0	3 3 2	3 2 1
	Mean 24 – 72 h	1	0	2.6	2

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

Eye irritation study by *Leuschner, 2006,* of Makhteshim Chemical Works Ltd., Beer-Sheva, Israel:

Eye irritation

Report:	KIIIA1 7.11/05, Leuschner, J., 2006
Annex II data point:	IIA 5.2.5
Title:	Acute eye irritation study of MCW 465 in rabbits
Testing facility:	LPT, Hamburg, Germany
Document No:	19778/06, Sponsor report no. R-20273
Guidelines:	OECD 405 (2002), EC method B.5 (2004/73/EC) Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary
A single application of 100 mg of the undiluted test item MCW 465 (99 % fluazinam) was placed into the conjunctival sac of the right eye of three male Himalayan rabbits. The left eye served as control. 24 hours after instillation the eyes were washed with 0.9 % aqueous NaCl solution. Ocular damage and irritation was assessed according to the OECD and EC guidelines 1 hour, 24, 48 and 72 hours and 4 to 6 days after treatment.
Administration of the test item caused reversible corneal opacity grade 1 in all animals 24 hours to 4 days after instillation, conjunctival redness grade 1 – 2 in all animals 1 hour up to 4 or 5 days (in 1 animal) after instillation and conjunctival chemosis grade 1 – 2 in all animals 24 hours to 72 hours or 4 days (in one animal) after instillation. No iridal effect was noted. In addition secretion was observed in all animals 1 hour to 48 hours after instillation. The eyes appeared normal 6 days after treatment.

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

M1	24 hours 48 hours 72 hours 4 days 5 days 6 days	1 1 1 1 0 0	0 0 0 0 0 0	2 2 2 1 1	2 2 2 1 0 0
M2	1 hour	0	0	1	0
	24 hours 48 hours 72 hours 4 days 5 days 6 days	1 1 1 1 0 0	0 0 0 0 0 0	2 2 1 1 0	2 2 1 0 0 0
M3	1 hour	0	0	1	0
	24 hours 48 hours 72 hours 4 days 5 days 6 days	1 1 1 1 0 0	0 0 0 0 0 0	2 2 1 1 0 0	2 2 1 0 0 0

4.4.2.2 Human information

No data.

4.4.2.3 Summary and discussion of eye irritation

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 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.
Considering the criteria for classification and labelling according to DIR 67/548/EEC and REG 1272/2008 in the eye irritation study by *Leuschner, 2006*, fluazinam was not irritating to the eyes.

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 Eye Dam. 1 - H318 and labeled with signal word “Danger”, 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 Eye Dam. 1 - H318 and labeled with signal word “Danger”.

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 3 - H335 Specific target organ toxicity –single exposure 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 STOT SE 3 - H335 Specific target organ toxicity –single exposure and labeled with signal word “Warning”.

4.5 Corrosivity

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

4.6 Sensitisation

4.6.1 Skin sensititsation

4.6.1.1 Non-human information

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*
Dermal sensitisation M & K-test	Guinea pig (Dunkin Hartley): Not sensitizing	Non-sensitising	*Chevalier, 2006*

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.

Skin sensitisation

Report:	KIIIA1 7.11/06, Chevalier, F., 2006
Annex II data point:	IIA 5.2.6
Title:	Examination of MCW 465 in the skin sensitisation test in guinea pigs according to Magnusson and Kligman (Maximisation Test)
Testing facility:	LPT, Hamburg, Germany
Document No:	19779/06, Sponsor report no. R-20274
Guidelines:	OECD 406 (1992), EC method B.6 (96/54/EEC) Deviations: none
GLP:	Yes (certified laboratory)

Executive Summary

The skin sensitisation properties of MCW 465 (99.0 % fluazinam) suspended in sesame oil were investigated in 10 Dunkin Hartley guinea pigs using the Magnusson and Kligman maximisation test. A vehicle control group of 5 animals was run concurrently. The treatment regime involved induction of sensitisation by intradermal injection on day 1, topical induction on day 8 (occlusive for 48 hours) and challenge by topical application on day 22 (occlusive dressing for 24 hours). Under the conditions of this study, none of 10 test group animals showed a dermal reaction after challenge with 0.5 % MCW 465 suspended in sesame oil.

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 by *Cummins, 1984,* and in the Buehler-Test by *Pritchard, 1986,* fluazinam caused evidence of delayed contact hypersensitivity 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.

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 Skin Sens.1A - H317 and labeled with signal word “Warning”, 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 Skin Sens.1A - H317 and labeled with signal word “Warning”.

4.6.2 Respiratory sensitisation

Based on a lack of data, fluazinam is not classified for this endpoint.

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 DIR 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.

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.

IKF-1216 Bacterial mutation assay

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

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

The study is considered acceptable.

Material and method:

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

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

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

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

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

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

(a) If treatment with a test substance produced an increase in revertant colony numbers of at least twice the concurrent solvent controls, with some evidence of a positive dose-relationship, in two separate experiments, with any bacterial strain either in the presence or absence of S-9 mix, it was considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.
(b) If treatment with a test substance did not produce reproducible increases of at least 1.5 times the concurrent solvent controls, in either mutation test, it was considered to show no evidence of mutagenic activity in this test system. No statistical analysis is performed.
(c) If the results obtained failed to satisfy the criteria for a clear "positive" or "negative" response given in paragraphs (a) and (b), additional testing may have been performed in order to resolve the issue of the test substance's mutagenic activity in this test system. Should an increase in revertant colony numbers then be observed which satisfies paragraph (a), the substance is considered to show evidence of mutagenic activity in this test system. No statistical analysis is performed.
If no clear "positive" response was obtained, the test data may have been subjected to analysis to determine the statistical significance of any observed increases in revertant colony numbers.

Findings:

In the first test, toxicity (visible thinning of the background lawn of non-revertant cells) was observed towards all tester strains at 50 µg/plate and above and towards all the *S. typhimurium* strains at 15 and 5 µg/plate in the absence of S-9 mix. In the second mutation test, toxicity was observed towards all tester strains at the highest dose level tested, 50 µg/plate, and towards all the *S. typhimurium* strains at 15 and 5 µg/plate in the absence of S-9 mix. Toxicity also was observed towards TA100 at 1.5 and 0.5 µg/plate in the absence of S-9 mix. In the third mutation test, toxicity was observed towards all tester strains at 50 and 15µg/plate and towards all the *S. typhimurium* strains at 0.5 µg/plate in the absence of S-9 mix. Toxicity also was observed towards TA100 in the presence of S-9 mix at 0.5µg/plate. No precipitation was observed in any test.
No evidence of mutagenic activity was seen at any dose level of fluazinam in any mutation test. The concurrent positive controls demonstrated the sensitivity of the assay and the metabolizing activity of the liver preparations, inducing substantial increases in revertant colony numbers with all strains.

Conclusion:

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

Bacterial reverse mutation test of fluazinam technical

Reference: *Ohtsuka M.; 1988;* Report No.T-1674E
Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.
The study is considered acceptable.

Material and method:

This test was conducted to evaluate the mutagenic potential of technical fluazinam (Lot No. 8412-20, purity 95.3%) in bacterial systems. The *S. typhimurium* (TA100, TA1535, TA98 and TA1537) test was performed with 0.0625, 0.125, 0.25, 0.5, 1 and 2 µg/plate in a DMSO solution without metabolic (S-9) activation and with 3.13, 6.25, 12.5, 25, 50 and 100 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). The *E. coli* (WP2 uvr A) test without S-9 mix was performed with 15.6, 31.3, 62.5, 125 and 250 µg/plate, and 31.3, 62.5, 125, 250 and 500 µg/plate with S-9 mix. Dose levels were established on the basis of preliminary range finding tests: Without metabolic (S-9) activation, fluazinam at dose levels of 1 and 3 µg /plate and above caused growth inhibition in *S. typhimurium strains* TA100 and TA98 respectively and with metabolic (S-9) activation at dose levels of 90 µg /plate and above. In *E. coli,* growth was inhibited at a dose of 250 µg /plate without metabolic (S-9) activation and at a dose of 500 µg /plate with metabolic (S-9) activation.
Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-

furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.
Evaluation criteria:
The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose response.
Findings:
The results of the tests showed that the number of revertant colonies for the tester strains exposed to fluazinam, at all dose levels, either with or without metabolic activation, were less than twice that for the solvent control. AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix (manifestation of revertant colonies for all bacterial strains).
Conclusion:
The results of this test indicate that fluazinam was not mutagenic in the bacterial reverse-mutation assays at the concentrations tested.

Bacterial reverse mutation test of fluazinam technical
Reference: *Ohtsuka M.; 1989;* Report No.T-1673E
Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.
The study is considered acceptable.
Material and method:
This test was conducted to evaluate the mutagenic potential of technical fluazinam (Lot No.109, purity 95.3%) in bacterial systems. The *S. typhimurium* (TA100, TA1535, TA98 and TA1537) test was performed with 0.0313, 0.0625, 0.125, 0.25, 0.5 and 1µg/plate in a DMSO solution without metabolic (S-9) activation and with 3.13, 6.25, 12.5 25, 50 and 100 µg/plate with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). The *E. coli* (WP2 uvr A) test without S-9 mix was performed with 15.6, 31.3, 62.5, 125 and 250 µg/plate, and with 31.3, 62.5, 125, 250 and 500 µg/plate with S-9 mix. Dose levels were established on the basis of preliminary range finding tests: Without metabolic (S-9) activation, fluazinam at a dose level of 1 µg /plate and above caused growth inhibition in *S. typhimurium strains* TA100, TA98 and TA 1535 and with metabolic (S-9) activation at a dose level of 100 µg /plate and above. In *E. coli,* growth was inhibited at a dose of 250 µg /plate without metabolic (S-9) activation and at a dose of 500 µg /plate with metabolic (S-9) activation.
Materials used as positive controls in the absence of S-9 mix were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN_3), and 2-methoxy-6-chloro-9-(3-(2-chloroethyl)-aminopropylamino) acridine 2 HCl (ICR-191); 2-aminoanthracene (2-AA) was used as the positive control in the presence of S-9 mix. Plates were incubated with the test substance for 48 hours at 37 °C and then counted for the number of revertant colonies. Duplicate plates were counted at each dose level.
Evaluation criteria:
The assay was considered positive if there was at least a two-fold increase in the mean number of revertants per plate, and the increase was accompanied by a dose-response.
Findings:
The results of the tests showed that the number of revertant colonies for the tester strains exposed to fluazinam, at all dose levels, either with or without metabolic activation, were less than twice that for the solvent control. AF-2, NaN_3 and ICR-191, used as positive controls, showed mutagenicity in the absence of S-9 mix, and 2-AA was mutagenic for all the strains in the presence of S-9 mix

(manifestation of revertant colonies for all bacterial strains).

Conclusion:

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

IKF-1216 Mammalian cell mutation assay

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

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

The study is considered acceptable.

Material and method:

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

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

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

Preliminary toxicity testing: A cell suspension was prepared in a 1:1 mixture of R10p and conditioned media. The cell suspension was placed on a roller apparatus for 30 minutes, and then 3 ml aliquots of the suspension were dispensed into sterile universal tubes. R0 or S-9 mix (2 ml) was added to each culture. Cultures (one with and one without S-9 mix) were prepared for each concentration of test compound. For treatment in the absence of S-9 mix using a continuous treatment over 24 hours, a cell suspension was prepared in R10p. Test substance was diluted to provide serial concentrations that were then incorporated into the cell suspensions. Fifty microliters of test substance or solvent were added to each suspension. The final concentrations of the test substance in the culture medium were 4.69, 9.38, 18.75, 37.5, 75, 150, 300, 450 and 600µg/ml. Cultures were placed on the roller apparatus for 3 or 24 hours at 37 °C. The cells were then washed with R10p and resuspended in 20 ml of R10p and counted on a Coulter Counter. A series of dilutions was then prepared and the cell cultures were plated and incubated for at least 7 days. The original cell suspensions were transferred into pre-gassed roller bottles and rolled for 48 hours. Suspension growth was monitored by sampling at 24-hour intervals. Cell density was counted using an electronic particle counter. After the initial count at 24 hours after treatment, the cell density was adjusted using R10p. The number of colonies per plate was counted and the Day_0 relative survival was calculated. This estimate of toxicity was then used to determine the concentrations of test substance to be used in the main tests.

Due to excess toxicity, a second preliminary test in the absence of S-9 mix using both the 3 hour treatment period and a continuous treatment period for 24 hours was carried out. The final

concentrations of the test substance in the culture medium were 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, 10 and 20 µg/ml.
Main test**:** Cell suspensions were prepared as in the toxicity testing with minor modifications. The size of the aliquots of cell suspensions, the amount of medium, and the amount of test substance or solvent added to the tubes were double the size used in the toxicity testing. The number of cultures prepared was doubled, two with and two without S-9 mix were prepared for each concentration of test substance. Throughout the main tests, toxicity was measured in terms of Day_0 relative survival (RS), and not suspension growth or relative total growth. The cultures were treated with 0.05, 0.1, 0.5, 1, 1.5, 2, 3, 4 and 5 µg/ml in the absence of S-9 mix and 0.5, 1, 2.5, 5, 7.5, 10, 12.5, 15 and 20 µg/ml in the presence of S-9 mix. All cultures were returned to the roller apparatus for 3 hours.
The cells then were washed once, re-suspended and Day_0 relative survival (RS) was assessed. The remaining cell suspensions were transferred into pre-gassed roller bottles and rolled for 48 hours to allow for expression of the mutant phenotype. Suspension growth was monitored by sampling at 24 and 48 hours to assess growth in suspension. After 48 hours the cells were assessed for cloning efficiency (Day_2) and mutant frequency.
Cloning efficiency was assessed by plating in R10p. Mutant frequency was assessed by plating in selective medium. The plates were placed in a humidified incubator at 37° C in an atmosphere of 5% CO_2 in air.
As a negative result was obtained in the first test, a second test in the absence of S-9 mix using continuous treatment over 24 hours was carried out. The concentrations tested were 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml. A second test in the presence of S-9 was carried out using 3 hour treatment. The concentrations were 0.5, 1, 3, 5, 6, 7, 8, 9 and 10 µg/ml.
After plates were incubated for at least 7 days, for cloning efficiency, or 10-14 days, for mutant frequency, the number of empty wells was determined for each microtitre plate (P0). This figure was used to calculate cloning efficiency and mutant frequency.
Day_0 relative survival (RS) and Day_2 cloning efficiencies (CE) were calculated as follows:
P(0): number of empty wells/total wells
CE: InP(0)/number of cells per well
Cell count factor: Individual treated post-treatment cell count/ Mean control post-treatment cell count
Survival: CE x cell count factor
RS: Individual survival value x 10/Mean control survival value
Toxicity was expressed in terms of Day_0 relative survival and not suspension growth or relative total growth. The mutant frequency per 10^6 survivors was calculated as follows.
CE in selective medium/CE in non selective medium
Evaluation criteria:
The statistical significance of the data was analyzed by methods described by Robinson *et al.* (1989).
Criteria for a response were:
The demonstration of a statistically significant increase in mutant frequency following treatment with the test substance.
Evidence of a dose relationship, over at least two dose levels, in any increases in mutant frequency.
Demonstration of reproducibility in any increases in mutant frequency.
The observed increases in mutant frequency must lie outside the historical control range with a corresponding Day_0 RS of not less than 10%.
The main test was done twice to assess reproducibility of responses.
Findings:
Preliminary toxicity: Treatment with 4.69 - 600 µg/ml fluazinam in the presence of S-9 mix resulted in a Day_0 relative survival over the range of 54 - 0% compared to the solvent controls. In the absence of S-9 mix, excess toxicity was observed after treatment with 4.69 - 600 µg/ml fluazinam with both the 3 hour and 24 hour treatment period and the results of this test are not

reported. In the additional preliminary toxicity test with both a 3 hour and 24 hour treatment period, the resulting Day_0 relative survivals were 116 - 0% and 105 - 0%, respectively. Concentrations used in the main test were based on these data.

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

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

Main test – presence of S-9: Treatment of cells with 0.5-20 µg/ml in Test 1 and 0.5-10 µg/ml in Test 2 resulted in Day_0 relative survivals of 97-0% and 107-3%, respectively. Cultures treated with 2.5, 5, 7.5, 10 and 12.5 µg/ml in Test 1 and 1, 3, 6, 7 and 9 µg/ml in Test 2 were plated with and without TFT (selective agent) to permit measurement of the levels of cloning efficiency and induced mutation. The resulting Day_2 cloning efficiencies over this range were 86 - 1% in Test 1 and 134 - 38% in Test 2 relative to controls.

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

Table 16a Cytotoxicity and mutant frequency in mouse lymphoma cells (mean values)

Compound	**conc. (µg/ml)**		**mean cell survival (% of control)**		**cloning efficiency [1] (%)**		**Mean mutant frequency per 10^6 survivors [2]**	
	1st test	**2nd**	**1st test**	**2nd**	**1st test**	**2nd**	**1st test**	**2nd**
without S-9 mix								
DMSO	-	-	100	100	100	100	74	84
Fluazinam		0.005		97				
		0.01		100				
		0.02		127		89		77
	0.05	0.05	90	88		103		98
	0.1	0.1	89	63		90		108
		0.2		33		93		109
		0.3		8		62		264
		0.4		3				
	0.5	0.5	77	2				
	1.0		70		78		83	
	1.5		43		79		89	
	2.0		24		87		100	
	3.0		8		40		190	
	4.0		3		20		199	
	5.0		1					
Methylmethan-sulphonate	10	5.0	74	55	62	52	728**	1548**
with S-9 mix								
DMSO	-	-	100	100	100	100	96	136
	0.5	0.5	96	107				
	1.0	1.0	97	92		134		102
	2.5		39		77		105	
		3.0		70		93		114
	5.0	5.0	62	47	86		105	

Compound	conc. (µg/ml) 1st test	2nd	mean cell survival (% of control) 1st test	2nd	cloning efficiency [1] (%) 1st test	2nd	Mean mutant frequency per 10^6 survivors [2] 1st test	2nd
		6.0		32		76		191
		7.0		23		38		186
	7.5		21		67		153	
		8.0		14				
		9.0		15		111		225
	10.0	10.0	6	3	28		227	
	12.5		1		1		1174**	
	15.0		0					
	20.0		0					
Methyl-cholanthrene	2.5	2.5	96	76	43	93	1120**	808**

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

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

Chromosomal aberration test of fluazinam technical using cultured mammalian cells
Reference: *Kajiwara Y.; 1988;* Report No.T-1663E
Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.
The study is considered acceptable.

Material and method:
This study assessed the clastogenic potential of technical fluazinam (lot number 109, purity 95.3%, dissolved in DMSO) by means of *in vitro* chromosomal aberration tests using Chinese-hamster lung fibroblasts (CHL cells, obtained from the National Institute of Hygienic Sciences) in the absence or presence of metabolic activation (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). CHL cells preserved by freezing were defrosted and cultured (cell culture: 5 ml of 10 % NCS/MEM (Eagles minimum essential medium supplemented with 10 % newborn calf serum)). 3 days after incubation, a subculture was made and the CHL cells in the logarithmic growth phase (stimulated to divide by treatment with phytohaem-agglutinin) were used for the test.
The concentrations of fluazinam tested were 1, 2 and 4 µg/ml without metabolic activation and 2.375, 4.75 and 9.5 µg/ml with S-9 mix, based on growth inhibition tests with fluazinam at concentrations of 0, 0.25, 0.5, 1, 2, 2.5, 4, 5, 7.5, 8, 10, 15, 20, 25 and 50 µg/ml (without metabolic activation) and 0, 0.5, 1, 2, 5, 8, 10, 15, 20 and 50 µg/ml (with metabolic activation).
Without S-9 mix, the two-day-old cultures were incubated for 24 and 48 hours at 37 ° C with the test substance. Cells which received S-9 mix were treated with the test material for 6 hours at 37 ° C. After S-9 mix including the test substance had been removed, the dishes were rinsed, placed in fresh media and incubated for further 18 hours.
2 hours prior to the end of the incubation, 0.1 µg/ml colcemid was added in order to prepare microscope slides of chromosomes.
Metaphase cells were then harvested and prepared for cytogenetic analysis.
Non-treatment and a solvent treatment group (DMSO) served as negative controls. Mitomycin C (MMC, 0.05 and 0.025 µg/ml) and cyclophosphamide (CPA, 5 µg/ml) were used as positive controls for direct-method and metabolic activation, respectively.
Evaluation criteria:
The test substance was considered positive if the incidence of cells with aberrations was increased more than 10% in a dose-related manner, or if the incidence was reproducible for at least one of the test points.

Findings:

In the solvent treatment and non-treatment groups without metabolic activation, the incidences of cells with structural chromosomal aberrations including gaps were 0 % and 1 % respectively after 24 and 48 hour treatment. The positive control treated with MMC showed structural chromosomal aberrations at an incidence of 59 % after 24 hour treatment and 34 % after 48 hour treatment. With metabolic activation, the incidences of cells with structural chromosomal aberrations including gaps were 0.5 % and 1 % for the non-treatment and solvent treatment groups, respectively. The positive control treated with CPA showed structural chromosomal aberrations at an incidence of 51.5 %.
After treatment with fluazinam, the mean percentage of cells with structural chromosomal aberrations including gaps was in a range between 0 % and 2 % with or without metabolic activation. These results indicate negative mutagenic activity of the test substance.
Table 16b: Mean % of aberrant cells (including and excluding gaps)

Dose (µg/mL)	**Without metabolic activation**				**With metabolic activation**	
	24 hours		**48 hours**		**24 hours**	
	Excl. Gaps	**Incl. gaps**	**Excl. gaps**	**Incl. gaps**	**Excl. gaps**	**Incl. gaps**
1 µg/mL	0.5	0.5	0	0		
2 µg/mL	0.5	0.5	0	0		
2.375 µg/mL					1	1
4 µg/mL	1	1.5	1	1		
4.75 µg/mL					0	0.5
9.5 µg/mL					2	2
DMSO (solvent control)	0	0	0.5	1	1	1
Negative control	0.5	1	0	0	0	0.5
MMC (Mitomycin C)	58***	59***	29**	34**		
CPA (Cyclophosphamide)					50.5***	51.5***

** significantly different from controls at $p < 0.01$; *** significantly different from controls at $p < 0.001$

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

DNA repair test of fluazinam technical in Bacillus subtilis
Reference: *Ohtsuka M.; 1988;* Report No.T-1595E
Guideline: The study was conducted according to Japanese MAFF, NohSan No. 4200 (1985) and is in compliance with GLP.
The study is considered acceptable.
Material and method:
This test was conducted to evaluate the mutagenic potential of technical fluazinam (lot number 109 purity 95.3%) in the *Bacillus subtilis* [H17(rec^+) and M45(rec^-)]. Fluazinam was tested in the DNA repair assay (Spore method), at concentrations of 0.003, 0.01, 0.03, 0.1 and 0.3 µg/disk without metabolic activation (S-9 mix), and 0.3, 1, 3, 10 and 30 µg/disk with S-9 mix (S-9 mix: liver preparations from Aroclor 1254-induced adult male Sprague-Dawley rats). *B.subtilis* was incorporated into agar. Paper disks were soaked with 20 µl containing various amounts of fluazinam, placed on the agar and incubated for 24 hours at 37° C. For those plates tested with metabolic activation, the S-9 fraction was incorporated in the agar. The zone of growth inhibition was determined for each plate. DMSO was used as the solvent control. Positive control materials used were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) and 2-aminoanthracene (2-AA), and Kanamycin was used as a negative control.
Evaluation criteria:
The minimal inhibition concentrations, (MIC), MIC rec^+ and MIC rec^-, are obtained from regression analysis of the dose-response relationship. The test is considered positive if the index of DNA damage (MIC rec^+ / MIC rec^-) is 2 or higher.

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

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.
IKF-1216 technical: Micronucleus test in mice
Reference: *Matsumoto K.; 1999;* Report No. IET 98-0139
Guideline: The study was conducted according to OECD Guideline 474 (1997), U.S. EPA Health Effects Guidelines (1991) and Japanese MAFF, 59 NohSan No. 4200 (1985) and is in compliance with GLP.
The study is considered acceptable.
Material and method:
This study was conducted to assess the potential induction of micronuclei by fluazinam technical (Lot No. 8412-20, purity of 95.6%) in bone marrow cells of mice. In Micronucleus test I (time-course study), 5 male and 5 female mice were treated with a single oral dose of fluazinam (intragastric gavage) at a dose level of 2000 mg/kg bodyweight (a preliminary toxicity test had previously shown that a dose of 2000 mg/kg was tolerated; this level was therefore selected as an appropriate maximum for use in the micronucleus test.). Bone marrow smears were prepared at three sampling times, i.e., 24, 48 and 72 hours after administration and examined for the frequency of micronucleated polychromatic erythrocytes.
In Micronucleus test II (dose-response study), mice were treated per gavage with a single oral administration of fluazinam at dose levels of 500, 1000 and 2000 mg/kg bodyweight. The bone marrow smears were prepared once 24 hours after administration.
The negative control group received the vehicle, olive oil, and bone marrow smears were obtained from five male and five female animals 24, 48 and 72 hours after dosing.The positive control group was dosed with mitomycin C at 10 mg/kg bodyweight and bone marrow smears were prepared once 24 hours after administration.
One smear from each animal was examined using light microscopy. The frequencies of micronucleated polychromatic erythrocytes and the polychromatic erythrocyte ratios were analyzed. The frequencies of micronucleated polychromatic erythrocytes are shown as percentages of polychromatic erythrocytes with micronuclei among 2000 polychromatic erythrocytes. The polychromatic erythrocyte ratios as an indicator of hematopoesis are shown as percentages of polychromatic erythrocytes among 1000 erythrocytes scored.
Evaluation criteria:
The mean frequency of micronucleated polychromatic erythrocytes in the negative control group should be 0.3% or less. The mean frequency of micronucleated polychromatic erythrocytes in the positive control group should be 2.0% or more.
Unless there is a substantial difference in response between sexes, results for the two sexes are combined to facilitate interpretation and maximize the power of statistical analysis.
A positive response is indicated if statistically significant dose-response increases in the frequency of micronucleated polychromatic erythrocytes are observed.

Findings:
In the time course study (micronucleus test I), no adverse clinical signs were observed in treated and control mice for the duration of the test. There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes at any sampling time of the fluazinam group either for males and females separately or combined. In the positive control group (mitomycin C), an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes. Statistically significant differences in the polychromatic erythrocyte ratio were observed in the fluazinam group but not the positive control group at the 24 and 48 hour sampling times. When these values (55.9% and 50.5%) were compared to the value of the vehicle control group at the 72 hour sampling time (53.6%), there were no differences between these values.
In the dose response study (micronucleus test II), in the fluazinam treated groups loose stool or external genital region soiled fur was observed in nine male mice. These signs were not observed in the positive and negative control groups.
There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes at any dose of fluazinam either for males and females separately or combined. In the positive control group treated with mitomycin C, an apparent increase was observed in the frequency of micronucleated polychromatic erythrocytes. Statistically significant decreases in the polychromatic erythrocyte ratios were not observed at any dose level of fluazinam or in the positive control group treated with mitomycin C.

Table 16c: Frequencies of micronucleated polychromatic erythrocytes (MNPCE/PCE) and polychromatic erythrocyte ratios (PCE/(PCE+NCE)

Sampling time	**Treatment**	**Dose (mg/kg)**	**MNPCE/PCE (%) (mean)**	**PCE/(PCE+NCE) (%) (mean)**
24 hours	Vehicle control	-	0.15 (test I) 0.16 (test II)	51.0 (test I) 53.6 (test II)
	Fluazinam	500	0.18	58.5
		1000	0.13	56.6
		2000	0.17 (test I) 0.15 (test II)	55.9*(test I) 51.5 (test II)
	Mitomycin C	10	3.30 (test I) 2.43 (test II)	47.3 (test I) 54.7 (test II)
48 hours	Vehicle control	-	0.14	59.0
	Fluazinam	2000	0.17	50.5*
72 hours	Vehicle control	-	0.14	53.6
	Fluazinam	2000	0.12	55.8

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

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

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.

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
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.
For completeness, tumour incidences in the 2 studies are presented.

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

Reference: *Mayfield R. et al; 1988;* Report No. ISK 8/87263
Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.
The study is in compliance with GLP.

Table17a: Incidences (%) of microscopic findings of male and female rats after 104 weeks of treatment (group mean values)

	0 ppm	1 ppm	10 ppm	100 ppm	1000 ppm

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

B-1216: Toxicity to Rats by Dietary Administration for 2 Years:
Reference: *Chambers P. R. et al; 1993;* Report No. ISK 43/920649
Guideline: The study was conducted according to Japanese MAFF Test Guidelines (1985), U.S. EPA Guidelines and OECD Guideline No. 452 and is in compliance with GLP. The study is considered acceptable.

Table 17b: Selected Pathology Findings (%) in Rats after 104 weeks of treatment

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

Testes:								
Tubular atrophy (total)	64		64		44		64	
Tubular atrophy (marked)	24		20		16		36	

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. For completeness, tumour incidences in the 2 studies are presented as well as data on mortality in the second study.
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.

B-1216: Potential Carcinogenicity Study in Dietary Administration to Mice for 104 Weeks:
Reference: *Mayfield R. et al; 1988;* Report No. ISK 9/87264
Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.
The study is in compliance with GLP.

Table17c: Incidences of liver cell tumours in male mice after 104 weeks of treatment

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

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

Table17d: Huntingdon Research Centre Ltd. historical control data for liver tumours in male mice for the years 1981 – 1983

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

	Study number								
Number of mice examined	52	104	104	52	88	52	52	52	52

Potential Tumorigenic Effects in Prolonged Dietary Administration to Mice:
Reference: *Chambers P. R. et al; 1998;* Report No. ISK 50/950671 and Addendum 1
Guideline: No specific test guideline is mentioned in the study, nevertheless, the study is considered acceptable.
The study is in compliance with GLP.

Table: 17e: Incidence and Percentage of mortality

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

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

Table17f: Incidence of hepatocellular tumors in male mice (expressed in percentages)

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

**: significantly different from control: p<0.01

Table 17g: Huntingdon Research Centre Ltd. historical control data for liver tumours in male mice for the years 1991 – 1993 (expressed in percentages)

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

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.

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 fetuses ↓; 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 estrus cycle, mating performance and fertility were recorded. Offspring was observed for clinical signs and mortality and body weights were recorded on days 1, 4, 7, 11, 14 and 21 after birth. Physical development was assessed on a litter basis based on pinna unfolding, hair growth, tooth eruption and eye opening.
Mating performance: vaginal smears were taken each morning following pairing and examined for the presence of spermatozoa. The day on which evidence of mating was found was designated day 0 of gestation.
Parameters were calculated as follows:
Percentage mating: animals mating/animals paired X 100
Conception rate: animals achieving a pregnancy/animals mating X 100
Fertility index: number of live litters born/number of pregnant females X 100
Gestation index: animals achieving a pregnancy/animals paired X 100
Gestation length: taken as the time between the day of successful mating and the day on which pups were first seen.
Specified organs from all F_0 and F_1 parental animals (liver, ovaries, prostate with seminal vesicles, testes with epididymides and uterus) were weighed. Histopathological examinations were performed on these organs and on vagina, pituitary (animals of suspect fertility) and on all abnormalities from control and high dose animals of the F_0 and F_1 generation. Examination was extended to the livers of males from the lowest and intermediate dietary concentration groups. Mammary tissue from any female which showed total litter loss was also examined microscopically.

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

Table 18a: 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.

Table 18b: 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 cull)	9.0	8.3	8.3	8.5
mean pup weight (g) day 21 p.p. (postcull)	53.5	51.7	52.0	48.4***
Pinna unfolding, completion (day p.p.)	3.3	3.4	3.5	3.1
Hair growth, completion (day p.p.)	3.0	3.3	3.3	3.3
Tooth eruption, completion (day p.p.)	10.5	11.2	10.8	10.7
Eye opening, completion (day p.p.)	14.7	14.7	14.5	13.8**

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

Table 18c: 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.

Table 18d: 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 fetuses removed by caesarean section. A gross macroscopic examination was performed and

specimens of tissues considered abnormal were retained. Liver and lungs were retained from all animals. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. All fetuses were dissected and examined internally.

Findings:

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

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

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

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

Table 18e: Reproduction data for does treated with fluazinam (mean group values)

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

Table 18f: 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 fetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. Liver and lungs were retained from all animals. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. All fetuses were dissected and examined internally. Placentae were weighed and examined for external abnormalities.

Findings:

The general condition of the treated females was similar to that of the controls throughout the study. Mean food consumption of animals treated with 7 and 12 mg/kg bw/d fluazinam was reduced throughout the dosing period, statistically significant during the second half of the dosing period. In the 4 mg/kg bw/d group, food consumption was reduced during the second half of the dosing period too, but statistical significance was not reached. At 2 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.
Absolute maternal body weights in animals dosed at concentrations of 2, 4 and 7 mg/kg/day were comparable to controls. Mean body weights in 12 mg/kg/day dosed animals were lower than concurrent controls from day 10 through Day 20 of gestation, reaching statistical significance on day 20. The body weights were increased during the postdosing period and the animals had recovered approximately 50% of their body weight losses by termination.

Table 18g: Mean maternal body weight (kg) during gestation

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

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

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

Table 18h: Microscopic Findings in the liver (16 animals/dose group) of does

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

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

Table 18i: Reproduction data for female rabbits treated with fluazinam (mean group values)

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

Preimplantation loss was elevated in all treated groups in comparison to the concurrent controls, but all values fell within the recorded background control range of the laboratory (4.7 – 35.7 % in 92 studies). Postimplantation loss was increased at 4 mg/kg/day compared to concurrent controls,

however, no increase was observed at the 7mg/kg/day dose level. A significant postimplantation loss was noted for the 12 mg/kg bw/d group. Fetal and placental weights were similar in all groups to concurrent control responses. There was a complete litter loss for 5 high-dose females and for one of the 7 mg/kg/day dose groups. No complete litter loss could be observed in controls and 2 and 4 mg/kg/day dose groups.

Table 18j: Group mean litter data for female rabbits treated with fluazinam

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

There were several abnormalities noted in fetuses during the external and visceral examination of all treatment groups, but mainly in the high-dose group. Several findings in the high-dose group were found only in a single litter or were within the historical control range. However, for placental anomalies (not nearer specified), the incidence was above the historical control range for the laboratory and appears to be due to treatment.
The incidence of several skeletal abnormalities was clearly increased in the high-dose group over both the study control values and the historical control range for the laboratory. Effects that may be treatment related include kinked tail tip, fused or incompletely ossified sternebrae and abnormalities of the head bones.

Table 18k: Percentage of fetal observations at skeletal examination (number of litters)

	Dose (mg/kg/day)					
Parameter	0	2	4	7	12	Historical control data (range)
Fetuses (litters)	136(15)	95(13)	63(10)	72(10)	44(7)+	86 - 92 studies 8407 –9385 fetuses
Placental anomalies	0.7(1)	3.2(3)	0.0	0.0	18.2(3)+	0.0 – 16.3
Head: additional sutures, parietal bones	0.7(1)	0.0	3.2(2)	2.8(2)	6.8(3)+	0.0 – 3.3

	Dose (mg/kg/day)					
Parameter	0	2	4	7	12	Historical control data (range)
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 fetuses removed by caesarean section. A gross macroscopic examination was performed and specimens of tissues considered abnormal were retained. The reproductive tract was dissected out and the number of corpora lutea, implantation sites, resorption sites and number of live and dead fetuses recorded. Fetuses were removed, sexed, weighed and examined externally for gross abnormalities. Fetuses were dissected and examined internally. Placentae were weighed and examined for external abnormalities.

Findings:
14 animals in the high dose group (250 mg/kg bw/d fluazinam) showed urogenital staining during the dosing phase. In other respects, the general condition of the treated females was similar to that of the controls throughout the study. Mean food consumption of animals treated with 250 mg/kg bw/d fluazinam was reduced statistically significant during the early dosing period. In the 50 mg/kg bw/d group, food consumption was reduced during the early part of the dosing period too, but statistical significance was not reached. At 10 mg/kg bw fluazinam, food consumption was similar in comparison to the concurrent control values throughout the study.
Animals dosed at concentrations of 250 mg/kg/day showed weight loss between days 6 and 8, followed by a slight reduced rate of weight gain between days 9 and 11 post coitum comparable to controls. Their rate of weight gain became slight superior to that of controls, although the overall weight gain from day 6 to 15 and to day 20 remained significantly reduced. Weight gain in the 50 mg/kg bw/d group was marginally, but not statistically significant, reduced. At 10 mg/kg bw fluazinam, weight gain was unaffected.

Table 18l: Mean maternal body weight gains (g) during gestation

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

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

Necropsy findings: Macroscopic examination of dams on day 20 of gestation revealed no changes attributable to treatment.
Reproduction data: Numbers of implantations, live young and the extent of preimplantation loss were unaffected by treatment with fluazinam. Postimplantation loss was increased in the 250 mg/kg/day group compared to concurrent controls, however, not statistically significant and within the range of the historical controls of the laboratory. Fetal and placental weights were significantly reduced in the high dose group. In the 50 mg/kg bw/d group, fetal and placental weights were clearly reduced compared to controls. 10 mg/kg/day dose groups were unaffected by treatment with fluazinam.

Table 18m: 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
g n	**Viable young**	13.8	13.5	14.3	13.4	11.1 – 14.8	10.9 – 15.9
i f i	**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
a n	**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 fetuses with an abnormality.

Table 18n: Incidences of facial/palatal clefts and/or incomplete ossification of palatine bones and diaphragmatic hernia in fetuses of high dose animals (250 mg/kg bw/d)

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

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

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

Table 18o: Percentage of fetal observations at skeletal examination (number of litters)

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

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

Conclusion:

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

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 fetuses, 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. Fetuses 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 fetuses 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.

Table 18p: 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)

Table 18q: 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 fetuses 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.

Table 18r: 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
s	**Implantation sites**	15.9	15.6	16.4	15.6
i	**Viable fetuses**	96.4	94.4	93.3	85.8*
g	**Resorptions total**	3.6	5.6	6.7	13.9
n	**early**	3.6	5.6	6.0	11.5
i	**late**	0.0	0.0	0.7	2.4
f	**Preimplantation loss (%)**	7.6	9.0	3.5	8.1
i c	**Postimplantation loss (%)**	3.6	5.6	6.7	14.2*
a	**Fetal weight (g)**	3.6	3.5	3.4*	3.0**
n t	**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) fetuses (litters) in the control, 10, 50 and 300 mg/kg bw/d groups, respectively.
Two fetuses of the 300 mg/kg bw/d group had a bent tail (0.6 %), one of them a bilateral microphthalmia (0.3 %). Two fetuses 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 fetuses, 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) fetuses (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.

Table 18s: 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)
Fetuses (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 fetuses.
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 fetuses.
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 fetuses, 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 fetuses.
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 Repr. 2 - H361 and labeled with 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, postimplantation loss, resulting in a statistically significant decrease of viable fetuses 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 Repr. 2 - H361 and labeled with signal word “Warning”.

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.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 19: Summary of relevant information on degradation

(Annex point as reference to the DAR)	Method	Results	Remarks	Reference
B.8.4.1.1 Hydrolysis rate (IIA 2.9.1)	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 -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 Degradation products: CAPA (5-chloro-6-(3-chloro- □,□,□-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. CAPA was steadily hydrolyzed to DCPA with a DT50 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.	Acceptable	van der Gaauw, A. (2003) (Document 846211)
B.8.4.1.2 Direct phototrans-formation (IIA 2.9.2)	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)

<table>
<tr><th>(Annex point as reference to the DAR)</th><th>Method</th><th>Results</th><th>Remarks</th><th>Reference</th></tr>
<tr><td>B.8.1.3
Photolysis in soil</td><td>US EPA, subdiv. N, 161-3</td><td>Degradation rates were recalculated by the RMS on the basis of single 1st order kinetics for the two label positions separately. The DT50 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). Under both, light and dark conditions conversion to bound residues was the main pathway. Metabolite HYPA as formed with max. 6.8% (day 28)</td><td>Acceptable</td><td>Lentz, N.R., Korsch, B.H. (2001</td></tr>
<tr><td>B.8.4.2
Biological degradation (A II 7.2.1.3.2)</td><td>Ready biodegradability
Manometric Respirometry Test OECD 301F; EU EEC, C.4-D</td><td>fluazinam is not readily biodegradable.</td><td>Acceptable</td><td>Lentz, N.R., Korsch, B.H. (2001</td></tr>
<tr><td>B.8.4.2.1
Water/Sediment Study (A II 7.2.1.3.2)</td><td>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)</td><td>
<table>
<tr><td colspan="4">Water/sediment system (simulation test) active substance Fluazinam</td></tr>
<tr><td></td><td>Phenyl label DT50</td><td>pyridyl label DT50</td><td>average both labels DT50</td></tr>
<tr><td>Virginia” water (pH 6.9)</td><td>1.93 d</td><td>2.85 d</td><td>2.4 d</td></tr>
<tr><td>“Emperor” water (pH5.6)</td><td>1.84 d</td><td>4.25 d</td><td>3.0 d</td></tr>
<tr><td>“Virginia” sediment (pH 6.6)</td><td>2.42 d</td><td>3.35 d</td><td>2.9 d</td></tr>
<tr><td>“Emperor” sediment (pH5.8)</td><td>6.41 d</td><td>9.5 d</td><td>7.9 d</td></tr>
<tr><td>“Virginia” whole system</td><td>3.3 d</td><td>2.93 d</td><td>3.1 d</td></tr>
<tr><td>“Virginia” whole system</td><td>5.23 d</td><td>6.2 d</td><td>5.7 d</td></tr>
<tr><td colspan="4">Water/sediment system (simulation test) Metabolite AMPA</td></tr>
<tr><td></td><td>phenyl label DT50</td><td>pyridyl label DT50</td><td>average both labels DT50</td></tr>
<tr><td>“Emperor” sediment (pH5.8)</td><td>24.0 d</td><td>43.7 d</td><td>33.9 d</td></tr>
</table>
</td><td>Acceptable</td><td>Goodyear 1997 (Report No. 38/188-1015)</td></tr>
</table>

(Annex point as reference to the DAR)	Method	Results	Remarks	Reference
B.8.1.1.1 Aerobic degradation in soil	Bharti & Bewick 1985 : no guideline Mawad: SETAC (Europe), 1995, part 1 and OECD Draft Guideline ("Aerobic and Anaerobic Transformation in Soil Systems"), 2000	Aerobic degradation in soil were calculated on basis of single 1st order kinetics and DT50 were in the range of 17 and 263 days. 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 CO2) 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 NO2 groups, respectively, on the phenyl ring of fluazinam, were found in minor amounts.		Bharti & Bewick 1985 Mawad 2003

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
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.

Photolysis in soil
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).

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

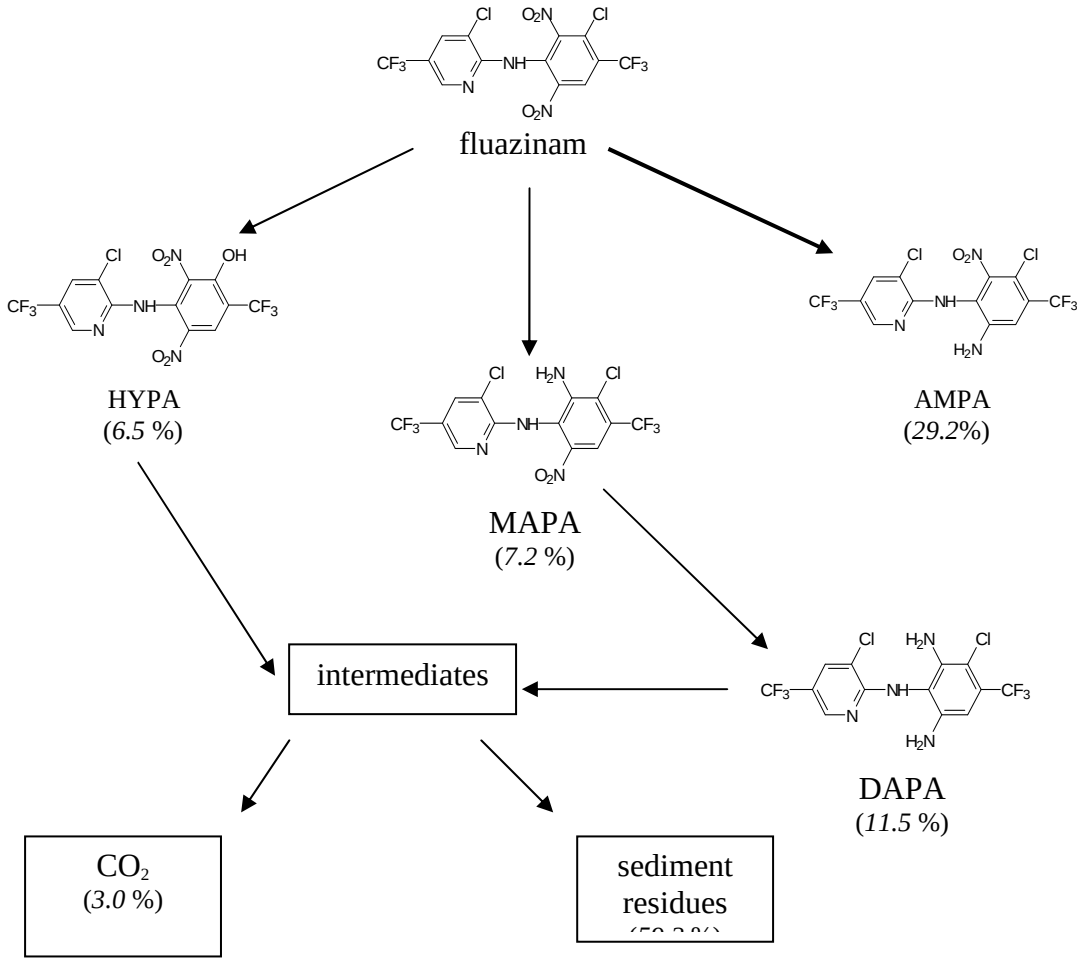
EXT.R-sed: extractable residues in sediment
NER: non extractable residues in sediment
nd: not detected
xx*: total unknowns, mixture of several polar compounds where individual substance did not exceed 2 % AR

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.



Proposed metabolic pathway of fluazinam in water/sediment systems

(***values in bracketc: maxima observed in the whole system for a single label position***

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	
"Virginia" 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.

Soil degradation

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.

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.

5.1.3 Summary and discussion of degradation

<table>
<tr><td colspan="4">Summary: Degradation</td><td>Test guideline / design</td><td>GLP (y/n)</td><td>Reliability</td></tr>
<tr><td colspan="7">Biotic degradation (% degradation in 28 davs (or, if absent, half- life in water (d)):</td></tr>
<tr><td colspan="4">Ready biodegradability
Fluazinam is not readily biodegradable under test conditions within 28 days (pH 7.4)</td><td>OECD 301F;
EU EEC, C.4-D</td><td>y</td><td>y</td></tr>
<tr><td colspan="4">Water/sediment system (simulation test) active substance fluazinam</td><td rowspan="11">BBA Guidelines, Part IV, Section 5-1; proposed UK Guidelines for the Conduct of Biodegradability Tests on Pesticides in Natural Sediment-Water Systems (1992)</td><td rowspan="11">y</td><td rowspan="11">y</td></tr>
<tr><td></td><td>phenyl label DT50</td><td>pyridyl label DT50</td><td>average both labels DT50</td></tr>
<tr><td>“Virginia” water (pH 6.9)</td><td>1.93 d</td><td>2.85 d</td><td>2.4 d</td></tr>
<tr><td>“Emperor” water (pH5.6)</td><td>1.84 d</td><td>4.25 d</td><td>3.0 d</td></tr>
<tr><td>“Virginia” sediment (pH 6.6)</td><td>2.42 d</td><td>3.35 d</td><td>2.9 d</td></tr>
<tr><td>“Emperor” sediment (pH5.8)</td><td>6.41 d</td><td>9.5 d</td><td>7.9 d</td></tr>
<tr><td>“Virginia” whole system (pH 6.6)</td><td>3.3 d</td><td>2.93 d</td><td>3.1 d</td></tr>
<tr><td>“Virginia” whole system (pH5.8)</td><td>5.23 d</td><td>6.2 d</td><td>5.7 d</td></tr>
<tr><td colspan="4">Water/sediment system (simulation test) Metabolite AMPA</td></tr>
<tr><td>AMPA: “Emperor” sediment</td><td>phenyl label DT50</td><td>pyridyl label DT50</td><td>average both labels DT50</td></tr>
<tr><td>(pH5.8)</td><td>24.0 d</td><td>43.7 d</td><td>33.9 d</td></tr>
<tr><td colspan="4">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 CO2 was very low. 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.</td><td></td><td></td></tr>
<tr><td colspan="4">Degradation in soil
Aerobic degradation in soil were calculated on basis of single 1st order kinetics and DT50 were in the range of 17 and 263 days. The main metabolic pathway is the formation of bound residues and HYPA was the major metabolie. Mineralization (formation of CO2) amounted for up to 6 % applied radioactivity after one year under standard conditions.</td><td>y</td><td>n</td></tr>
</table>

Abiotic degradation			
Hydrolysis: Fluazinam may be considered hydrolytic stable under acidic conditions, under neutral conditions it is rapidly hydrolysed wit dt50 values in the range 2.7 – 4.5 d <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. CAPA was steadily hydrolyzed to DCPA with a DT50 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.	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	y	y
Photolysis DT50 = 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 14CO2 (17.7% and 16.0% of the phenyl and pyridyl labels, respectively after 30 days)		y	y

Discussion:

Fluazinam it is rapidly hydrolysed with dt50 values in the range 2.7 – 4.5 under neutral conditions. The major metabolite CAPA was steadily hydrolyzed to DCPA with a DT50 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. Based on this data primary degradation could be indicated, but ultimate degradation could not shown in this study .

Rapid aquatic photolytic degradation was shown to occur with dt50 = 2.5d. 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 CO2 production. The only major metabolite for both labels is G-504 (max. 17.1 % after 10 days). CO2 production was max. 17.7 % at day 30, indicating low ultimate degradation.

Ready biodegradability
After 28 days the BOD in the test flasks was 12 and 14 mg O2/L (arithmetic mean 13 mg O2/L). The biodegradation rate was 1 % based on $ThOD_{NH4}$ and 0 % based on $ThOD_{NO3}$. Therefore fluazinam is not ready biodegradable.

In water/sediment studyFluazinam was rapid degraded with a DT50 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 DT50 values of 43.7 days (pyridyl label; "Emperor" sediment).

The mineralization to CO2 was low with maximal amounts of 2.2 % at day 100 indicating very low ultimate degradation.

Conclusion:

Fluazinam is not readily biodegradable under 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) and G-504 (metabolite formed in photolysis)

-the proposed classification (R53, H413) of metabolite AMPA

a non rapid degradation is proposed.

5.2 Environmental distribution

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

Not relevant to classification

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	BCFss whole fish phenyl label 1090* / pyridyl 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. BCF was determined only for viscera and fillet, but not for lipid (lipid content was not specified in the DAR).

5.3.2 Summary and discussion of aquatic bioaccumulation

Summary

	• Test guideline / design	pH	GLP (y/n)	Reliability
At 25 °C (99.4% w/w) **log P_{OW}** 4.19 (pH 4 to 7) 3.5 (pH 8) 2.5 (pH 9)	Partition coefficient 40 CFR 158.190 Pesticide Assessment Guidelines Subdivision D: Product Chemistry Guideline 63-11	4 -7 8 9	y	y
Bioconcentration				
BCFss whole fish	Bioconcentration EPA Guideline 165-4		y	y
• phenyl label • 1090*				
• pyridyl label • 960*				
* based on total ^{14}C residues				

Discussion:

The log K_{ow} is > 4, indicating a potential for bioaccumulation and exceeds the two classification criteria of 3 and 4,.

Fluazianm exhibits moderate bioaccumulation in fish. The BCF of 960 - 1090 exceeds the two classification criteria of 100 and 500.

Note: In DAR BCF was determined only for viscera and fillet, but was not corrected by lipid content.

5.4 Aquatic toxicity

Table 35: Summary of relevant information on aquatic toxicity

Method	Results for the active substance Fluazinam							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	Measured pH 6.8– 7.1	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 for the active substance Fluazinam							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	Measured pH 6.7–7.6	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

Table 36: Summary of relevant information on aquatic toxicity

Method	Results for the Metabolite AMPA (water solubility limit: < 0.04 mg/L)							Remarks	Reference
	test organism	test condition	time	endpoint	test conc.	NOEC (µg ai/l)	LOEC (µg ai/l)		
OECD 203	*Brachydanio rerio* Zebra fish	static	96 hr	mortality	m	≥ 90	**> 90**		Hertl, A. (1997a)
OECD 202	*Daphnia magna* Waterflea	static	48 hr	immobility	m	≥ 260	**> 260**		Hertl, A. (1997b)
OECD 201	*Scenedesmus subspicatus* Green alga	static	72 hr	biomass growth rate	m	≥ 240	**> 240**		Hertl, A. (1997c)

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.

Acute toxicity to fish (IIA 8.2.1)
Reference: Hertl, A. (1997a): Acute Toxicity of AMPA to Zebra Fish (*Brachydanio rerio*) in a 96-Hour Static Test. Report No. 662512
Test guideline: OECD 203
GLP: yes
Test item: AMPA 98.7 % w/w, Lot No.: 9511
Material and methods:
The acute toxicity of the metabolite AMPA to zebra fish (*Brachydani rerio*) was assessed in a 96 hours static test. Due to the low solubility of the test substance (< 0.04 mg test substance/L) fish were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark), dilutions of 1:2, 1:4, 1:8, and 1:16 and an untreated control. Seven fish were tested for each concentration. At the start of the test the fish had a mean body length of 3.0 ± 0.15 cm and a mean body wet weight of 0.26 ± 0.03 g. The water temperature, pH and dissolved oxygen concentrations of test media were measured daily and reported as follows: 23 – 24°C, pH 7.5 – 7.9 and ≥ 8.1 mg O_2/L. Chemical analysis of test item concentrations was carried out only for the undiluted stock solution at 0, 48 and 96 hours of the exposure period.
Findings:
The measured concentrations in filtrate (highest concentration level) were: 0.131 mg/L at 0 hr, 0.084 mg/L at 48 hr and 0.063 mg/L at 96 hr, respectively. The mean measured concentration was 0.09 mg/L (calculated as the average over all measured concentrations) and was well above the maximal water solubility of 0.04 mg/L.
In all concentrations (filtrate with 0.09 mg/L and 1:2, 1:4, 1:8, 1:16 dilutions) and the control no sublethal effects and mortalities were observed until the end of the study. Therefore the NOEC is ≥ 0.09 mg/L and the LC_{50} is > 0.09 mg/L.
Conclusion: LC_{50} (96 h): > 0.09 mg AMPA/L and NOEC: ≥ 0.09 mg AMPA/L based on mean measured concentrations of the filtrate of a supersaturated stock solution (solubility limit AMPA: 0.04 mg/L)
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 37: 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
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 38: 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) 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.

Acute toxicity to aquatic invertebrates (IIA 8.2.4)
Reference: Hertl, J. (1997b): Acute Toxicity of AMPA to *Daphnia magna* in a 48-Hour Immobilization Test. Report No. 662490
Test guideline: OECD 202
GLP: yes
Test item: AMPA, purity: 98.7 %, Lot No: 9511
Material and methods:
Young daphnids (less than 24 hours old) were exposed to AMPA (metabolite of fluazinam) under static conditions for 48 hours. Due to the low solubility of the test substance (< 0.04 mg test substance/L) daphnids were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark). The study was performed as a limit test with one concentration (using the filtrate) and a control. The test concentration was analytically determined in the test medium (undiluted filtrate) at the start and at the end of the test.
Test conditions: 20 ± 1 °C; 16 h light / 8 h dark photoperiod, pH 7.8 – 9.0; 7.8 – 8.9 mg/L dissolved O_2, a total hardness of 250 mg/L (as $CaCO_3$).
Findings:
At the initiation 0.28 mg/L and at termination 0.24 mg/L of test substance was measured. All biological endpoints were related to the mean measured concentration of 0.26 mg/L. During the exposure period no sublethal effects or immobility were observed in the control and at the tested concentration (0.26 mg/L). Therefore the NOEC was ≥ 0.26 mg/L and the EC50 > 0.26 mg/L:

Conclusion: EC_{50} (48 h): > 0.26 mg AMPA/L and NOEC: ≥ 0.26 mg AMPA/L based on mean measured concentrations of the filtrate of a supersaturated stock solution (solubility limit AMPA: 0.04 mg/L)
Comment (RMS): Study considered acceptable.

Acute toxicity to aquatic invertebrates (IIA 8.2.4)
Reference: Hertl, J. (1997b): Acute Toxicity of AMPA to *Daphnia magna* in a 48-Hour Immobilization Test. Report No. 662490
Test guideline: OECD 202
GLP: yes
Test item: AMPA, purity: 98.7 %, Lot No: 9511
Material and methods:
Young daphnids (less than 24 hours old) were exposed to AMPA (metabolite of fluazinam) under static conditions for 48 hours. Due to the low solubility of the test substance (< 0.04 mg test substance/L) daphnids were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark). The study was performed as a limit test with one concentration (using the filtrate) and a control. The test concentration was analytically determined in the test medium (undiluted filtrate) at the start and at the end of the test.
Test conditions: 20 ± 1 °C; 16 h light / 8 h dark photoperiod, pH 7.8 – 9.0; 7.8 – 8.9 mg/L dissolved O_2, a total hardness of 250 mg/L (as $CaCO_3$).
Findings:
At the initiation 0.28 mg/L and at termination 0.24 mg/L of test substance was measured. All biological endpoints were related to the mean measured concentration of 0.26 mg/L. During the exposure period no sublethal effects or immobility were observed in the control and at the tested concentration (0.26 mg/L). Therefore the NOEC was ≥ 0.26 mg/L and the EC50 > 0.26 mg/L:
Conclusion: EC_{50} (48 h): > 0.26 mg AMPA/L and NOEC: ≥ 0.26 mg AMPA/L based on mean measured concentrations of the filtrate of a supersaturated stock solution (solubility limit AMPA: 0.04 mg/L)
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.

Effects on algal growth and growth rate (IIA 8.2.6)
Reference: Hertl, J. (1997c): Toxicity of AMPA to *Scenedesmus subspicatus* in a 72-Hour Algal Growth Inhibition Test for Poorly Soluble Test Substances. Report No. 662477
Test guideline: OECD 201
GLP: yes
Test item: AMPA, purity: 98.7 %, Lot No: 9511
Material and methods:
The effects of AMPA on the growth of the unicellular green alga *Scenedesmus subspicatus* were assessed in a growth inhibition test (limit test). The algal cultures (1 x 10^4 cells/ml) were exposed to undiluted filtrate of a supersaturated stock solution (100 mg/L continuously stirred for up to 2 hours in the dark) and a control. Test samples (3 replicates of filtrate, 6 controls) were incubated for up to 72 hours under static conditions and continuous illumination. The temperature was 23 °C and the pH ranged from 7.9 – 8.1 at the start of the test. Cell densities were determined by electronic particle counter (Coulter Counter Model ZM). Chemical analysis of test item in the undiluted stock solution was carried out at the start and the end of the test.
Findings:
At the end of the test pH had increased to 10.7 due to the high CO_2 consumption of fast-growing algae.
At the initiation 0.25 mg/L and at termination 0.23 mg/L of test substance were measured. All biological endpoints were related to the mean measured concentration of 0.24 mg/L.
No toxic effects were observed in the highest tested concentration (filtrate with maximal dissolvable concentration of AMPA). Therefore the NOEC is ≥ 0.24 mg/L and the E_bC_{50}/E_rC_{50} (72 h) is > 0.24 mg/L.
Conclusion: E_bC_{50}/E_rC_{50} (72 h): > 0.24 mg AMPA/L and NOEC: ≥ 0.24 mg AMPA/L based on the mean measured concentration of the filtrate of a supersaturated stock solution (solubility limit of AMPA: 0.04 mg/L)
Comment (RMS): Study considered acceptable.

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.

Summary and discussion: Acute (short-term) aquatic toxicity

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/EC50 = 0.036 mg/l (measured pH 6.8 – 7.1)**				

Data element: Acute (short-term) aquatic toxicity of the Metabolite AMPA (main metabolite detected in water/sediment study) • 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}):				
• *Brachydanio rerio*	• > 0.090	• OECD 203	• y	• y
• Crustacea (48 hr EC_{50}):				
• *Daphnia magna*	• > 0.260	OECD 202	• y	• y
• Algae (72 E_rC_{50}):				
• *Scenedesmus subspicatus*	• > 0.240	OECD 201	• y	• y
Conclusion: "**No acute toxicity**" as L(E)C50s are above the water solubility. Due to the low solubility (< 1 mg/L) of the test substance the tests could not performed with higher test concentrations. No mortality, no sub-lethal effects or immobility or toxic effects were observed in the tested concentration.				

Summary and discussion: Chronic (long-term) aquatic toxicity

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 $NOEC_{F0\ growth,\ F1\ survival}$):				
• *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)				

Data element: Chronic (long-term) aquatic toxicity is based on acute (short-term) aquatic toxicity of the Metabolite AMPA (main metabolite detected in water/sediment study) (no chronic aquatic toxicity studies with fish or daphnia are available)				
•	• $L(E)C_{50}$ [mg/L]	• Test guideline / design	• GLP (y/n)	• Reliability
• Fish (96 hr LC_{50}):				
• *Brachydanio rerio*	• > 0.090	• OECD 203	• y	• y
• Crustacea (48 hr EC_{50}):				
• *Daphnia magna*	• > 0.260	OECD 202	• y	• y
• Algae (72 E_rC_{50}):				
• *Scenedesmus subspicatus*	• > 0.240	OECD 201	• y	• y
Conclusion: AMPA was poorly soluble and no acute toxicity is recorded at levels up to the water solubility. No mortality, no sub-lethal effects or immobility or toxic effects were observed in the tested concentration.				

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

Endpoint	Classifcation Criteria		Evidence for Fluazinam
	CLP (2nd ATP)	**DSD**	
Degradation Fluazinam	Fluazinam it is rapidly hydrolysed with DT50 values in the range 2.7 – 4.5 under neutral 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 was found to be photolytically unstable, with a DT50 of 2.5 days. 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 metabolite for both labels is G-504 (max. 17.1 % after 10 days). CO_2 production was max. 17.7 % at day 30, indicating low ultimate degradation. Fluazinam is not readily biodegradable under test conditions within 28 days (pH 7.4). In water/sediment studies Fluazinam was degraded with a DT50 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 DT50 values of 43.7 days (pyridyl label; "Emperor" sediment). 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 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) and G-504 (metabolite formed in photolysis) -the proposed classification (R53, H413) of metabolite AMPA* (metabolite formed in water/sediment study) **a non rapid degradation is proposed.** ***** (see below) chronic aquatic toxicity of metabolite AMPA
Bioaccumulation Fluazinam	**BCF > 500** (960 – 1090) **Log K_{ow} is > 4** (4.19 at pH 4 to 7)	**BCF > 100** (960 – 1090) **Log K_{ow} is > 3** (4.19 at pH 4 to 7)	The BCF* and the Log Kow exceeds the classification criteria for DSD as well as for CLP indicating a **potential for bioaccumulation.** *In DAR 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 .

Endpoint	CLP (2nd ATP)			Evidence for Fluazinam
	CLP (2nd ATP)		DSD	
Acute aquatic toxicity Fluazinam	**LC/EC$_{50}$ ≤ 1 mg/L**			
	Active substance Fluazinam			Fluazinam is of high acute toxicity to fish *(Oncorhynchus mykiss*) with a LC50 = 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 **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.
	Oncorhynchus mykiss		**LC50 = 0.036 mg**/L	
	Daphnia magna		EC50 = 0.220 mg/L	
	Pseudokirchn. Subcapitata		E$_r$C$_{50}$ = 0.220 mg/L	
Chronic aquatic toxicity Fluazinam	**For nonrapidly 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 $_{\text{F0 growth, F1 survival}}$ = 0.0029 mg/L. Therefore Fluazinam fulfills the criteria for the proposed classification as **H410** according to Regulation EC 1272/2008. **A M-factor of 10** is applicable based on 0.001 < NOEC ≤ 0.01 mg/l.
	Pimephales promelas	**NOEC$_{\text{F0 growth, F1 survival}}$ = 0.0029mg/L**		
	Daphnia magna	NOEC $_{\text{growth}}$ = 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 DT50 values of 43.7 days (pyridyl label; "Emperor" sediment).		Based on DT50 of 43.7 d in a water/sediment system **a non rapid degradation** of the metabolite AMPA is proposed
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)C50s are above the water solubility; Water solubility = <1 mg/L**		"**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 performed with higher test concentrations. No mortality, no sublethal effects or immobility or toxic effects were observed in the test concentrations.
	Brachydanio rerio	LC50 => 0.090 mg/L	
	Daphnia magna	EC50 => 0.260 mg/L	
	Scenedesmus subspicatus	E_rC_{50} = > 0.240 mg/L	
Chronic aquatic toxicity of metabolite AMPA	**L(E)C50s are above the water solubility ; Water solubility = <1 mg/L** (no chronic aquatic toxicity studies with fish or daphnia are available)		AMPA was poorly soluble and no acute toxicity is recorded at levels up to the water solubility. AMPA is not rapidly degradable (DT50 water/sediment = 43.7 d) and no experimentally determined BCF or log Kow 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 **H413** according to Regulation EC 1272/2008.
	Brachydanio rerio	LC50 => 0.090 mg/L	
	Daphnia magna	EC50 => 0.260 mg/L	
	Scenedesmus subspicatus	E_rC_{50} = > 0.240 mg/L	

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

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

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

Classification categories	aquatic environmental hazard **acute category 1** aquatic environmental hazard **chronic category 1**	
GHS Pictogram		
Signal Word	Warning	
Hazard Statement	H400 H410 EUH401	‘Very toxic to aquatic life’, ‘Very toxic to aquatic life with long lasting effects’ ‘To avoid risks to human health and the environment, comply with the instructions for use’
M-factor (acute) based on 0,01 < L(E)C50 ≤ 0,1 mg/l.		10
M-factor (chronic) based on 0.001 <NOEC ≤0.01 mg/l and the substance being not rapidly degradable.		10
Precautionary statements — Prevention	P273 P391 P501	Avoid release to the environment Collect spillage Dispose of contents/container to ….

6 OTHER INFORMATION

Environmental fate properties and environmental hazard assessments of this CLH report were based on studies and summaries based on the Draft Assessment Report and its addenda.

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**
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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
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Bramstedt, W. R., Kogovsek, L. M	1999	Characterization of B-1457 (IKF-1216 Impurity, Lot 9604). Ricerca, Inc., Report No. 4039-98-0177-AS-001 GLP: yes	Y	ISK
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	N	ISK

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		GLP: no unpublished		
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
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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
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van der Gaauw,	2003	14C-Fluazinam: Hydrolysis at Three Different	Y	ISK

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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
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7.2 Human health hazard assessment

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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
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Beck M.	IIA, 5.6.2	2006	B-1216: Teratology study in the rat Report No.: WIL-282006 GLP: yes Unpublished	Y	ISK

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Chevalier, F.	IIA, 5.2.6	2006	Examination of MCW 465 in the skin sensitisation test in guinea pigs according to Magnusson and Kligman (Maximisation test) LPT, Hamburg, Germany Report no. 19779/06, Sponsor report no. R-20274 GLP / GEP Unpublished	yes	MCW
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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
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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
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KIIIA1 7.11/05					

7.3 Environmental hazard assessment

7.3.1 Fate and Behaviour in the environment

Atkinson, R.	1993	Estimation of Hydroxyl Radical Reaction Rate Constants: Fluazinam. Ricerca Inc., Report No. RIC 1832 Not 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
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

Galicia, H., Völkl, S.	1991	Soil Adsorption/ Desorption of Fluazinam (IKF-1216) on Four Soils. RCC Umweltchemie AG, Report No. 282306 GLP, unpublished	N	ISK
Goodyear, A.	1997	^{14}C-Fluazinam: Biodegradation in Natural Water-Sediment Systems. Covance Laboratories, Report No. 38/188-1015 GLP, unpublished	N	ISK
Grützner, I.	2000	Ready Biodegradability of Fluazinam in a Manometric Respirometry Test. RCC Ltd, Report No. 774898 GLP, unpublished	Y	ISK
Gurney A.	2005a	Kinetic calculations for degradation of fluazinam in soil under laboratory and field conditions RCC Ltd report no. A07132, July 1, 2005 Not GLP, unpublished	Y	ISK
Kennedy, S.H.	1996	Fluazinam Soil Degradation Study Following Applications to Potatoes and Bare Ground (UK, 1995). CEM Analytical Services Ltd., Report No. CEMS-451 GLP, unpublished	N	ISK
Kennedy, S.H.	1996	Fluazinam Soil Degradation Study Following Applications to Potatoes and Bare Ground (UK, 1995). CEM Analytical Services Ltd., Report No. CEMS-451 GLP, unpublished	N	ISK

Lentz N.R., Korsch B.H.	1994	A photolysis Study of IKF-1216 in water at pH 5 (part 1) Ricerca, report no. 5312-94-0119-EF-001, Interim report, December 20, 1994 GLP, unpublished	N	ISK
Lentz, N.R., Korsch, B.H.	2001	A Photolysis Study of IKF-1216 (Fluazinam) on Soil. Ricerca, Inc., Amended Report No. 5313-95-0011-EF-002 GLP, unpublished	Y	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, unpublished	N	ISK
Mawad, N.	2003	Metabolism And Degradation Of ^{14}C-Fluazinam In One Soil Incubated Under Aerobic Conditions. RCC Ltd, Report No.844056 GLP, unpublished	Y	ISK
Mawad, N.	2003	Metabolism And Degradation Of ^{14}C-Fluazinam In One Soil Incubated Under Aerobic Conditions. RCC Ltd, Report No.844056 GLP, unpublished	Y	ISK
Muller, K., Lane, M. C. G.	1993	Fluazinam: Adsorption and Desorption Properties in Soil of R270682 (“HYPA”), a Major Soil Metabolite. ICI Plant Protection Division, Report No. RJ1308B GLP, unpublished	N	ISK

Ryan, J., Sapiets, A.	1992	Fluazinam: Laboratory Soil Degradation Study (BBA). ICI Agrochemicals, Report No. RJ1391B GLP, unpublished	N	ISK
van der Gaauw, A.	2002	Degradation Rate of HYPA in Three Soils Incubated Under Aerobic Conditions. RCC Ltd, Report No. 842279 GLP, unpublished	Y	ISK
van der Gaauw, A.	2003	^{14}C-Fluazinam: Hydrolysis at Three Different pH Values. RCC Ltd, Report No. 846211 GLP, unpublished	Y	ISK

7.3.2 Aquatic Toxicity

Fillmore, G. E. & J. Laveglia	1993	Technical Fluazinam (IKF-1216) – The Toxicity to Fathead Minnow (*Pimephales promelas*) During Early Life-Stage Exposure. Generated by: Springborn Laboratories Report No. 5018-91-0425-TX-002 GLP / GEP: yes unpublished	N	ISK

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

Hertl, J.	1997c	Toxicity of AMPA to *Scenedesmus subspicatus* in a 72-Hour Algal Growth Inhibition Test for Poorly Soluble Test Substances. RCC Umweltchemie AG Report No. 662477 GLP / GEP: yes unpublished	N	ISK
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

Peither, A.	2001b	Acute Toxicity of Fluazinam to Guppy (*Poecilia reticulata*) in a 96-Hour Flow-Through Test. Generated by: RCC Ltd, Switzerland, Report No: 813453 GLP / GEP: yes unpublished	Y	ISK
Sankey, S. A., Tapp, J. F., Caunter, J. E., Stanley, R. D.	1992	Fluazinam: The 28 Day LC50 to Rainbow Trout (*Oncorhynchus mykiss*). Generated by: ICI Brixham Laboratory Report No. BL4167/B GLP / GEP: yes unpublished	N	ISK
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
Shults, S. K., Brock, A. W., Laveglia, J.	1993	Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions with Technical Fluazinam (IKF-1216). Generated by: Springborn Laboratories, Report No. 5109-91-0419-TX-002 GLP / GEP: yes unpublished	N	ISK
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