Committee for Risk Assessment

RAC

Annex 1

Background document

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

Fenpyrazamine

EC number: NA

CAS number: 473798-59-3

CLH-O-0000001412-86-55/F

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

Adopted

12 March 2015

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Fenpyrazamine

EC Number: ***Not allocated***

CAS Number: ***473798-59-3***

Index Number: **-**

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Austria

Version number: 2 **Date: June 2014**

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Fenpyrazamine***
EC number:	***Not allocated***
CAS number:	***473798-59-3***
Annex VI Index number:	**-**
Degree of purity:	***Minimum purity 94.0 % w/w (based on a pilot plant)***
Impurities:	***No relevant impurities***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Aquatic Acute 1, H400, M = 10 Aquatic Chronic 1, H410, M = 10
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Acute 1, H400, M = 10 Aquatic Chronic 1, H410, M = 10

1.3 Proposed harmonised classification and labelling based on CLP Regulation

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	-		-	Conclusive, but not sufficient for classification
2.9.	Pyrophoric liquids	-		-	Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids	-		-	Conclusive, but not sufficient for classification
2.11.	Self-heating substances and mixtures	-		-	Conclusive, but not sufficient for classification
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	-		-	Hazard class not assessed in this dossier
	Acute toxicity - dermal	-		-	Hazard class not assessed in this dossier
	Acute toxicity - inhalation	-		-	Hazard class not assessed in this dossier
3.2.	Skin corrosion / irritation	-		-	Hazard class not assessed in this dossier

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.3.	Serious eye damage / eye irritation	-		-	Hazard class not assessed in this dossier
3.4.	Respiratory sensitisation	-		-	Hazard class not assessed in this dossier
3.4.	Skin sensitisation	-		-	Hazard class not assessed in this dossier
3.5.	Germ cell mutagenicity	-		-	Hazard class not assessed in this dossier
3.6.	Carcinogenicity	-		-	Hazard class not assessed in this dossier
3.7.	Reproductive toxicity	-		-	Hazard class not assessed in this dossier
3.8.	Specific target organ toxicity –single exposure	-		-	Hazard class not assessed in this dossier
3.9.	Specific target organ toxicity – repeated exposure	-		-	Hazard class not assessed in this dossier
3.10.	Aspiration hazard	-		-	Hazard class not assessed in this dossier
4.1.	Hazardous to the aquatic environment	H400 H410	M = 10 M = 10	-	-
5.1.	Hazardous to the ozone layer	-		-	Conclusive, but not sufficient for classification

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

Labelling: Signal word: Warning!
Pictogram: GHS9
Hazard statements: H400 (M = 10), H410 (M = 10)

Proposed notes assigned to an entry:

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Fenpyrazamine is currently approved to be used as an active substance in plant protection products (Commission Implementing Regulation (EU) No 595/2012).

This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of fenpyrazamine under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID 5 dossier.

Austria submitted a CLH proposal to ECHA in December 2011 and after evaluating acute toxicity, specific target organ toxicity – single exposure (STOT SE), respiratory and skin sensitisation, specific target organ toxicity (CLP) and repeated dose toxicity (DSD) – repeated exposure (STOT RE), germ cell mutagenicity, carcinogenicity, reproductive toxicity and environmental hazards, RAC adopted an opinion on the proposal in November 2012 concluding that fenpyrazamine should be classified as Aquatic Chronic 2; H411. However, this classification and labelling is not yet included in Annex VI and will be included in the 6th ATP to CLP later in 2014.

In connection with a product registration for the central zone the RMS became aware of additional Annex II studies with marine species, but also with freshwater species. These studies were submitted by the notifier Sumitomo for the re-assessment of the classification and labelling. None of these studies were submitted for Annex I inclusion of the active substance fenpyrazamine. Under consideration that the acute and chronic endpoints are adverse compared to the available data a revised CLH report was submitted. No other registration dossiers are available for fenpyrazamine at time of the submission of the revised CLH report.

Fenpyrazamine is not listed on Annex VI of the CLP Regulation This proposal seeks for classification for environment. No classification is required for human health and physico-chemical properties.

2.2 Short summary of the scientific justification for the CLH proposal

For Fenpyrazamine, no classification and labelling has been proposed regarding physical and chemical properties and human health.

Justification for the proposal with respect to environmental effects:

The classification and labelling of the active substance Fenpyrazamine is based on the high acute and chronic toxicity to aquatic invertebrates (marine), bivalves and algae (marine and freshwater diatoms) and the fact that the active substance is not rapidly biodegradable (Lewis, C.J. & Troth, K., 2007). The log P_{ow} of fenpyrazamine is 3.52 (Lentz, N.R., 2005b).

Combing all these criteria for classification with respect to environmental effects, according to Regulation 1272/2008, *H400 Very toxic to aquatic life* and *H410, Very toxic to aquatic life with long lasting effects,* is proposed for Fenpyrazamine.

2.3 Current harmonised classification and labelling

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

No current entry in Annex VI, Table 3.1 in the CLP Regulation.

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

No current entry in Annex VI, Table 3.2 in the CLP Regulation.

2.4 Current self-classification and labelling

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

No current self-classification based on the CLP Regulation criteria.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification for pesticides.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

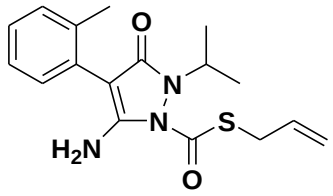
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	-
EC name:	-
CAS number (EC inventory):	-
CAS number:	473798-59-3
CAS name:	1H-pyrazole-1-carbothioic-acid, 5-amino-2,3-dihydro-2-(1-methylethyl)-4-(2-methylphenyl)-3-oxo, *S*-2-propen-1-yl ester
IUPAC name:	S-allyl 5-amino-2-isopropyl-4-(2-methylphenyl)-3-oxo-2,3-dihydro-1H-pyrazole-1-carbothioate
CLP Annex VI Index number:	-
Molecular formula:	$C_{17}H_{21}N_3O_2S$
Molecular weight range:	331.43 g/mol

Structural formula:



1.2 Composition of the substance

Table 5: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Fenpyrazamine	940 g/kg	Minimum purity, no range	The minimum purity based on a pilot plant and should be considered provisionally. If commercial production launches (2012) a different minimum purity might be specified.

Current Annex VI entry: no entry

Table 6: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No relevant impurities	-	-	-

Current Annex VI entry: -

Table 7: Additives (non-confidential information)

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

Current Annex VI entry: -

1.2.1 Composition of test material

Physico-chemical properties: see table 9 (purity of tested technical material in the range from 94.7% to 99.3%)

Human health hazard assessment: purity of tested technical material either 94.7% (all toxicological studies performed with the same batch) or 99.4% (ADME studies only).

Environmental hazard assessment: purity of tested technical material in the range from 94.7 % to 99.3 %.

1.3 Physico-chemical properties

Table 8: Summary of physico - chemical properties:

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	OECD 102 Equivalent to EEC Method A.1 DSC GLP	PGAI R-4CM03G 99.3%	Melting point: 116.4 °C (389.6 K)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.2 Boiling point (IIA 2.1.2)	US EPA OPPTS 830.7220 – Boiling point Equivalent to EEC Method A.2 DSC and capillary GLP	PGAI R-4CM03G 99.3%	Boiling point: 239.8 °C (513.0 K) at a nominal pressure of 745 mm/Hg	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	US EPA OPPTS 830.7220 – Boiling point Equivalent to EEC Method A.2 GLP	PGAI R-4CM03G 99.3%	No decomposition was observed. Individual melting and boiling points determined, therefore no sublimation occurred.	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
B.2.1.4 Relative density (IIA 2.2)	US EPA OPPTS 830.7300 – Density Equivalent to EEC Method A.3 Pycnometer method GLP	PGAI R-4CM03G 99.3%	Relative density at 20 °C: 1.262	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
		TGAI 030-050914-1G 94.7%	Relative density at 20 °C: 1.250	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.5 Vapour pressure (IIA 2.3.1)	OECD 104 EEC Method A.4 – gas saturation method and calculation (MPBPWin) GLP	PGAI R-4CM03G 99.3% *)	Vapour pressure by gas saturation method: $<10^{-5}$ Pa at 25 °C (too low to be determined experimentally). Vapour pressure by MPBPWin calculation: 2.89×10^{-8} Pa at 25 °C.	Acceptable According to EEC A.4 calculated values can be used if the vapour pressure is likely $<10^{-5}$ Pa at ambient temperature. The calculation confirms the low	DiFrancesco, D., 2006 (QNP-0004)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
				value. *) The original study reveals a purity of > 98%. The certificate of analysis confirms the purity of 99.3%.	
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	calculation	--	The Henry's Law Constant at 20 ˚C is calculated to be 1.62 x 10^{-4} Pa.m^3/mole. (Calculated from vapour pressure of 10^{-5} Pa and water solubility of 20.4 mg/L at 20 °C.)	Acceptable	Document M II Section 1
B.2.1.7 Appearance: physical state and colour (IIA 2.4.1)	US EPA OPPTS 830.6302 - Color / ASTM D-1535 US EPA OPPTS 830.6303 - Physical State GLP	PGAI R-4CM03G 99.3%	White, Munsell reference: N9.5/90% at 21.7 °C Solid at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.6302 - Color / ASTM D-1535 US EPA OPPTS 830.6303 - Physical State GLP	TGAI 030-050914-1G 94.7%	Very pale yellow, Munsell reference: 10Y 9/2 at 20.7 °C Solid at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.8 Appearance: odour (IIA 2.4.2)	US EPA OPPTS 830.6304 - Odor GLP	PGAI R-4CM03G 99.3%	Slight odour at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.6304 - Odor GLP	TGAI 030-050914-1G 94.7%	Odour characteristic of garlic at 25 °C	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)

<table>
<tr><th>Property (Annex point as reference to the DAR)</th><th>Method</th><th>Material / Batch</th><th>Results</th><th>Conclusion/Comment</th><th>Reference (Study)</th></tr>
<tr><td>B.2.1.9.1
Spectra of the active substance [UV/VIS]
(IIA 2.5.1.1)</td><td>US EPA OPPTS 830.7050
OECD 101
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>UV/Vis: Spectra in 90/10 v/v/ water/methanol measured in acidic (addition of aqueous HCl), unadjusted and basic solutions (addition of aqueous NaOH).

<table><tr><th>Solution</th><th>λ_{max} (nm)</th><th>ε [L x · cm^{-1} x mol^{-1}]</th></tr><tr><td rowspan="2">Acidic
pH 1.4-1.5</td><td>243</td><td>16600</td></tr><tr><td>274</td><td>13800</td></tr><tr><td rowspan="2">Unadjusted
pH 7.8-8.1</td><td>243</td><td>16700</td></tr><tr><td>274</td><td>13900</td></tr><tr><td>Basic
pH 12.7</td><td colspan="2">No maxima due to decomposition in basic medium.</td></tr></table></td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.2
Spectra of the active substance [IR]
(IIA 2.5.1.2)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>IR spectrum provided and consistent with the structure of S-2188.

<table><tr><th>cm^{-1}</th><th>Assignment</th></tr><tr><td>3423</td><td>N-H stretch</td></tr><tr><td>2970, 2932</td><td>C-H stretch</td></tr><tr><td>1668</td><td>C=O stretch</td></tr></table></td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.3
Spectra of the active substance [NMR]
(IIA 2.5.1.3)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>^{1}H and ^{13}C NMR spectra provided and consistent with the structure of S-2188.
This is demonstrated by the chemical shift peak assignments.</td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.4
Spectra of the active substance [MS]
(IIA 2.5.1.4)</td><td>US EPA OPPTS 830.7050
GLP</td><td>PGAI
R-4CM03G
99.3%</td><td>Electron Impact Mass Spectrum (EI/MS) provided and consistent with structure of S-2188.</td><td>Acceptable</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
<tr><td>B.2.1.9.5
Wavelengths at which UV/VIS molecular extinction occurs, where appropriate, to include a wavelength</td><td></td><td>PGAI
R-4CM03G
99.3%</td><td>Measurements up to 750 nm show no more absorptions as reported in B.2.1.9.1.</td><td>Acceptable
No absorption above 290 nm.</td><td>Sweetapple, G.G. & Lentz, N.R., 2006a
(QNP-0006)</td></tr>
</table>

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
at the highest absorption above 290 nm (IIA 2.5.1.5)					
B.2.1.9.6 Optical purity (IIA 2.5.1.6)			Not relevant since active substance is no resolved isomer		
B.2.1.10 Spectra of relevant impurities (IIA 2.5.2)			Not relevant since no impurities of toxicological or environmental concern are stated.		
B.2.1.11 Solubility in water (IIA 2.6)	Japanese MAFF (12-Nousan-No. 8147, Part 2-9-8, 2000) OECD 105, US EPA OPPTS 830.7840, and EEC Method A6 shake flask method; determination HPLC GLP	PGAI R-4CM03G 99.3%	Water solubility at neutral pH at 20 °C: 20.4 mg/L The effect of pH on water solubility was not determined as S-2188 does not dissociate under acidic or basic conditions.	Acceptable	Lentz, N.R., 2005a (QNP-0003)
B.2.1.12 Solubility in organic solvents (IIA 2.7)	US EPA OPPTS 830.7840 OECD 105 determination HPLC GLP	PGAI R-4CM03G 99.3%	n-hexane: 902 mg/L n-octanol: 84403 mg/L (99174 mg/kg) toluene: 112978 mg/L (126297 mg/kg) acetone: > 250 g/L (> 250 g/kg) methanol: > 250 g/L (> 250 g/kg) dichloromethane: > 250 g/L (>250 g/kg) ethyl acetate: > 250 g/L (> 250 g/kg)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)
	US EPA OPPTS 830.7840 OECD 105 determination HPLC GLP	TGAI 030-050914-1G 94.7%	n-hexane: 811 mg/L n-octanol: 99223 mg/L (105230 mg/kg) toluene: 129308 mg/L (132262 mg/kg) acetone: > 250 g/L (> 250 g/kg) methanol: > 250 g/L (> 250 g/kg) dichloromethane: > 250 g/L (>250 g/kg)	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
			ethyl acetate: > 250 g/L (> 250 g/kg)		
B.2.1.13 Partition coefficient *n*-octanol/water (IIA 2.8.1)	Japanese MAFF (12-Nousan-No. 8147, Part 2-9-11, 2000) OECD 107 US EPA OPPTS 830.7550 EEC Method A8 shake flask method GLP	PGAI R-4CM03G 99.3%	n-octanol/water partition coefficient: 3307.32 log Pow = 3.52 at 25 ± 1 ºC (pH: 7.2)	Acceptable	Lentz, N.R., 2005b (QNP-0002)
Effect of pH (4-10) on the n-octanol/water partition co-efficient (IIA 2.8.2)			The effect of pH on partition coefficient was not determined as S-2188 does not dissociate under acidic or basic conditions.		
B.2.1.16 Quantum yield (IIA 2.9.3)	Japanese MAFF (12-Nousan-No. 8147, Part 2-6-2, 2000) EPA Pesticide Assessment Guidelines, Sub-division N, Section 161-2 SETAC Procedures for Assessing the Environmental Fate and Ecotoxicity of Pesticides, Section 10 GLP	[Phenyl-^{14}C] S-2188 batch number CFQ 14367 Radio-chemical purity: 99.4% [Pyrazolyl-5-^{14}C] S-2188 batch number CFQ 14368 Radio-chemical purity: 99.4%	Quantum yield in pH 7 buffer: 0.021	Acceptable	Lewis, C.J. & Troth, K., 2007d (QNM-0029)
B.2.1.17 Lifetime in the top layer of aqueous systems (calculated and real) (IIA 2.9.4)	Japanese MAFF (12-Nousan-No. 8147, Part 2-6-2, 2000) EPA Pesticide Assessment Guidelines, Sub-division N, Section 161-2 SETAC Procedures for Assessing the	[Phenyl-^{14}C] S-2188 batch number CFQ 14367 Radio-chemical purity: 99.4% [Pyrazolyl-5-^{14}C] S-2188	Photodegradation in sterile water at pH 7 and 25 ºC in summer sunlight at UK/US conditions(ca 25 Watt/m2 at 300-400 nm): **Label** / **DT_{50} (days)** / **DT_{90} (days)** Pyrazolyl / 1.7 / 5.5 Phenyl / 1.6 / 5.4	Acceptable	Lewis, C.J. & Troth, K., 2007d (QNM-0029)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
	Environmental Fate and Ecotoxicity of Pesticides, Section 10 GLP	batch number CFQ 14368 Radio-chemical purity: 99.4%			
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.5)	Japanese MAFF (12-Nousan-No. 8147, Part 2-9-14, 2000) US EPA OPPTS 830.7370 OECD 112 spectrophotometric method GLP	PGAI R-4CM03G 99.3%	In a pH screening experiment on S-2188, absorbance bands at 243 and 273 nm were observed in acidic and unadjusted 90/10 v/v water/methanol solutions (~ pH 1 and 7). A shift to lower wavelengths was observed in basic solution (~ pH 13). This shift was not reversible, indicating that the shift was due to basic decomposition rather than dissociation. No dissociation activity was observed in the approximate pH range 1 – 13.	Acceptable	Beckwith, R.C. & DiFrancesco, D., 2005 (QNP-0001)
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Atkinson calculation, performed using Atmospheric Oxidation programme EPIWIN (AOPWIN v 1.9) GLP	--	Photochemical reaction with OH radicals. Assuming a 12 hr day and a hydroxyl radical concentration of 1.5×10^6 OH/cm³ (EPA), decomposition half life was calculated to be 1.221 hrs. i.e. <2 days.	Acceptable	Liney, P. & Jarvis, T., 2009 (QNM-0032)
B.2.1.20 Flammability (IIA 2.11.1)	EEC Method A.10 GLP	TGAI 030-050914-1G 94.7%	In the preliminary screen S-2188 did not ignite. Not classified as flammable.	Acceptable according to Directive 67/548/EEC. The result is acceptable according to Regulation 1272/2008 as well. No classification.	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.21 Auto-flammability (IIA 2.11.2)	EEC Method A.16 - Relative self-ignition temperature GLP	TGAI 030-050914-1G 94.7%	S-2188 showed no exothermic reaction. Not auto-flammable. (Measurement up to 400 °C).	Acceptable according to Directive 67/548/EEC. According to the Regulation 1272/2008 no test procedure for self heating (N.4) is required if the substance is completely molten at 160 °C.	Weissenfeld,M., 2009 (QNP-0014)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
				This is demonstrated by DSC plots for determination of melting point and boiling point [Sweetapple, G.G. & Lentz, N.R., 2006a (QNP-0006)] No classification.	
B.2.1.22 Flash point (IIA 2.12)			Not required, as S-2188 does not melt below 40 ºC.		
B.2.1.23 Explosive properties (IIA 2.13)	US EPA OPPTS 830.6316 GLP	TGAI 030-050914-1G 94.7%	Preliminary thermal explodability screen: No evidence of explodability observed up to 200ºC. Impact explodability: No evidence of explodability at the maximum impact drop height.	Test is not according to EEC A14.	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
	Statement		Evaluation based on oxygen balance and structural consideration: The calculated oxygen balance is -205.2%. This value is considered to be outside of the potential for explosivity. The chemical structure does not indicate any potential for explosivity.	Statement is acceptable S-2188 (Fenpyrazamine) is considered having no explosive properties according to Directive 67/548/EEC. The statement is acceptable according to Regulation 1272/2008 as well. No classification.	Asada, Y., 2010 (QNP-0019)
B.2.1.24 Surface tension (IIA 2.14)	OECD 115 EEC Method A.5 GLP	TGAI 030-050914-1G 94.7%	Surface tension: 66.9 mN/m at a concentration of 90% of the saturation solubility and 20 ºC.	Acceptable	Sweetapple, G.G. & Lentz, N.R., 2006b (QNP-0007)
B.2.1.25 Oxidizing properties (IIA 2.15)	Statement according to EEC Method A17		An examination of the structure of S-2188 reveals that it contains none of the reactive groups or oxidizing compounds known to increase oxidizing power. It does contain some electronegative atoms (N, S, O), but these are bonded only to carbon and/or hydrogen, and therefore, are unlikely to add to the	Acceptable The statement is acceptable according to Regulation 1272/2008 as well.	Liney, P. & Jarvis, T., 2009 (QNP-0008)

Property (Annex point as reference to the DAR)	Method	Material / Batch	Results	Conclusion/Comment	Reference (Study)
			oxidizing power. The structural examination of S-2188 suggests it is not likely to possess oxidizing properties.	No classification.	
B.2.1.2.26 pH (IIA 2.16)			Not required for EU		
B.2.1.2.27 Storage stability (IIA 2.17.1)			Not required for EU		
B.2.1.2.28 Stability (temperature, metals) (IIA 2.17.2)			Not required for EU		
B.2.1.2.29 Other/special studies (IIA 2.18)			None		

According to Directive 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

Fenpyrazamine is a fungicide to be used for control of grey mould (*Botrytis)*. It is not systemic but there is some translocation in plants. Fenpyrazamine shows its fungicidal activity through inhibition on germ tube elongation and mycelium elongation. However, the biochemical mechanism of fungicidal activity is not clarified to date.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification required.

4 HUMAN HEALTH HAZARD ASSESSMENT

5 ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Fenpyrazamine is currently approved to be used as an active substance in plant protection products. In November 2012 RAC adopted an opinion that fenpyrazamine should be classified as Aquatic Chronic 2; H411. However, this was not considered as an existing entry in Annex VI by the dossier submitter (DS) at the time when the new CLH dossier was submitted to ECHA on 4 June 2014. The 6th Adaptation to Technical Progress (ATP) to the CLP Regulation was published in the Official Journal of the European Union on 6 June 2014, meaning it is currently listed in Annex VI of the CLP Regulation. Additional ecotoxicity data made available since the original opinion was adopted led to the submission of an updated CLH report to revise the environmental classification. The (revised) classification and labelling of fenpyrazamine is based on its high acute and chronic toxicity to aquatic invertebrates (marine), bivalves and algae (marine and freshwater diatoms) and the fact that the active substance is not rapidly degradable.

The DS proposed to classify fenpyrazamine as Aquatic Acute 1 (H400) with an M-factor of 10 and Aquatic Chronic 1 (H410) with an M-factor of 10, based on the lowest acute EC_{50} of 0.034 mg/L for growth rate of the alga *Skeletonema costatum* and the lowest chronic NOEC of 0.0049 mg/L based on yield for the alga *Navicula pelliculosa*.

Degradation

Fenpyrazamine is hydrolytically stable at 20°C at pH 4 and 7, but is rapidly hydrolysed at pH 9, with a half-life of 24 days. Aqueous photolysis is rapid with extensive breakdown after 30 days' incubation and an estimated half-life of 1.7 days at pH 7 and 25°C under

natural summer sunlight conditions, although photolysis is not relevant for classification.

The substance was degraded by an average of 1% after 28 days in a ready biodegradation test (OECD TG 301B). Simulation tests in two aerobic water-sediment systems using radio-labelled substance indicated primary degradation and formation of non-extractable residues, with first order degradation DT_{50} values for the whole system of 18 – 68 days (geometric mean 35.5 days), and relatively little mineralisation over 100 days (3.1 – 8.5% of applied radioactivity (AR)). Aerobic degradation in soils followed a similar pattern, with limited mineralisation after 120 days (5.2 – 8.5% of AR) and DT_{50} values of 24 – 40 days.
Based on the lack of ready biodegradation, limited mineralisation and primary degradation half-lives exceeding 16 days in an aquatic simulation study, fenpyrazamine does not meet the criteria for being rapidly degradable in the environment.

Bioaccumulation
The n-octanol/water partition coefficient (log K_{ow}) of fenpyrazamine is 3.5 at 25 °C and pH 7.2. The experimentally derived steady state bioconcentration factor (BCF) for the parent substance was between 8 and 9 L/kg wet weight (ww) for fish with an average lipid content of about 1.9% (w/w). This is equivalent to a BCF of up to 24 L/kg ww after normalisation to 5% lipid content. The parent substance was extensively metabolised in fish, and the steady-state BCF based on total radio-active residues (TRR) was 283 – 289 L/kg ww (equivalent to a BCF of up to 760 L/kg ww after normalisation to 5% lipid content). The major residues were the metabolite S-2188-DC and its glucuronic acid conjugate (at concentrations in whole fish of 8.0 – 18.8% and 16.1 – 33.3% TRR, respectively). More than 95% of the ^{14}C residues were eliminated during the depuration phase (within 14 days), and the depuration half-life was less than one day.

S-2188-DC is also one of the main products of photolysis, alkaline hydrolysis and mammalian metabolism. It forms through loss of the S-2-propen-1-yl carbothioic-acid ester group from the parent substance. No data are presented about the aquatic degradability of S-2188-DC (too few data were available in the water-sediment study to estimate a DT_{50}). In the DAR a log K_{ow} of 0.23 is reported (estimated using KOWWIN, version not stated). It is not stated whether this substance falls within the applicability domain of the model, but it appears to have a lower bioaccumulation potential than the parent. Aquatic acute toxicity tests for fish, *Daphnia* and algae are summarised in the DAR, and it is an order of magnitude less acutely toxic than the parent substance (all acute L(E)C_{50}s were above 82 mg/L; the 72-h NOEC for algae was 2.7 mg/L). Based on this evidence, fish metabolites do not need to be taken into account in defining the BCF for fenpyrazamine.

In summary the BCF for the parent substance is below 500 L/kg for the purposes of classification and labelling.

Aquatic Toxicity
Reliable acute and chronic aquatic toxicity data are available for the three trophic levels fish, aquatic invertebrates and algae. Based on the test with one of most sensitive algae *Skeletonema costatum*, RAC notes that despite the potential for photolysis, the concentration in the algal study was well maintained. The most sensitive organisms for both acute and chronic tests are as follows (the key study results are highlighted in bold):

Test organism	Short-term	Long-term
Oncorhynchus mykiss	96-h LC_{50} = 5.2 mg/L	90-d NOEC = 0.37 mg/L
Cyprinodon variegatus Sheepshead minnow	96-h LC_{50} > 3.9 mg/L	**33-d NOEC = 0.062 mg/L**

Daphnia magna	48-h EC_{50} = 5.5 mg/L	21-d NOEC = 0.34 mg/L
Americamysis bahia Mysid	**96-h EC_{50} = 0.83 mg/L**	**28-d NOEC = 0.024 mg/L**
Crassostrea virginica Oyster	**96-h EC_{50} = 0.66 mg/L**	
Pseudokirchneriella subcapitata	72-h E_rC_{50} > 0.9 mg/L	72-h NOE_rC = 0.22 mg/L
Navicula pelliculosa Freshwater diatom	**96-h E_rC_{50} = 0.202 mg/L**	**96-h NOE_rC = 0.074 mg/L** **96-h NOEC = 0.0049 mg/L (Yield)**
Skeletonema costatum Marine diatom	**96-h E_rC_{50} = 0.034 mg/L**	**96-h NOE_rC = 0.011 mg/L**

Acute toxicity
The DS proposed to classify fenpyrazamine as Aquatic Acute 1 (H400) based on acute toxicity to:

- the aquatic invertebrates *Americamysis bahia* (96-h LC_{50} = 0.83 mg/L based on immobility) and *Crassostrea virginica* (96-h EC_{50} = 0.66 mg/L based on shell deposition); and
- the algae *Navicula pelliculosa* (96-h E_rC_{50} = 0.202 mg/L) and *Skeletonema costatum* (96-h E_rC_{50} = 0.034 mg/L).

Chronic toxicity
The DS proposed to classify fenpyrazamine as Aquatic Chronic 1 (H410) based on long-term toxicity to:

- the fish *Cyprinodon variegatus* (33-d NOEC = 0.062 mg/L based on growth); the aquatic invertebrate *Americamysis bahia* (28-d NOEC = 0.024 mg/L based on growth); and the algae *Navicula pelliculosa* (96-h NOEC = 0.0049 mg/L based on yield and 0.074 mg/L based on growth rate).

Comments received during public consultation

Comments were received from four Member States (MS), who all supported the DS's proposal to classify fenpyrazamine as Aquatic Acute 1 and Aquatic Chronic 1, as well as the proposed acute M-factor of 10. Two MS queried the basis for the chronic M-factor (see below).

One commenter noted that the dossier did not include some additional valid data, but pointed out that this had no influence on the proposal. The DS replied that the study with the freshwater algae *Anabaena flos-aquae* was not included because it was not considered valid. The study for the sediment organism *Chironomus riparius* was included in the CLH report for the first submission of fenpyrazamine and was accidentally deleted for the revised submission.

One MS disagreed with the proposed chronic M-factor of 10 and suggested to use the 96-h NOE_rC of 0.011 mg/L for *Skeletonema costatum* as the most sensitive algal result instead of the 96-h NOEC of 0.0049 mg/L for *Navicula pelliculosa* based on cell density, as growth rate is the preferred endpoint for classification because it is independent of test design. Another MS asked for an explanation of why the yield endpoint should be used when a NOE_rC was available from the same study. In reply, the DS was of the opinion that the most sensitive endpoint should be used for chronic classification.

The CLP guidance (and the CLP Regulation), however, state that the classification shall be based on the ErC50, which also applies for the NOEC. RAC considers that the yield

endpoint (based on biomass measurement) suffers from similar statistical drawbacks as the biomass endpoint. The growth rate endpoint is therefore preferred when available. This is consistent with the CLP guidance for acute endpoints and also EFSA Guidance for plant protection products.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS's proposal to consider fenpyrazamine as not rapidly degradable, based on hydrolytic stability at pH 4 and 7, 1% degradation in a ready biodegradation test, and limited primary degradation (mean DT50 35.5 d) with minimal mineralisation in a water-sediment simulation study.

Bioaccumulation

RAC agrees with the DS's proposal that fenpyrazamine does not meet the CLP criteria for bioaccumulation, based on a parent BCF of 8-9 L/kg (up to 24 L/kg ww after normalisation to 5% lipid content).

Aquatic Toxicity

RAC notes that there are reliable acute and chronic aquatic toxicity data for fish, aquatic invertebrates and algae. The marine diatom *Skeletonema costatum* is the most sensitive species in both acute and chronic tests.

Based on the available information, RAC is of the opinion that fenpyrazamine should be classified as:

Aquatic Acute 1 based on a 96-h E_rC_{50} of 0.034 mg/L for *S. costatum*. As this value is above 0.01 mg/L but ≤ 0.1 mg/L, the **acute M-factor is 10**.

Aquatic Chronic 1 based on a 96-h NOE_rC of 0.011 mg/L for *S. costatum*. As this value is above 0.01 mg/L but ≤ 0.1 mg/L, and the substance is not rapidly degradable, the **chronic M-factor is 1**. RAC disagrees with the DS's proposed chronic M-factor of 10 based on a yield NOEC of 0.0049 mg/L for *N. pelliculosa*.

Conclusion on Classification

Fenpyrazamine should be classified as:
Aquatic Acute 1 (H400), M=10;
Aquatic Chronic 1 (H410), M=1.

This classification was based on the substance being not rapidly degradable, non-bioaccumulative and very toxic to aquatic organisms. RAC agrees with the DS's proposal with the exception of the chronic M-factor.

5.1 Degradation

Table 9: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: EEC Method C.7 , OPPTS 835-2110, J MAFF Nousan 8147, section 2-6-1 (2001)	Fenpyrazamine, [phenyl-14C] and [pyrazolyl-14C] labels: DT50 (pH 4, 20 °C): stable to hydrolysis DT50 (pH 7, 20 °C, extrapolated): > 1 year (SFO) DT50 (pH 9, 20 °C, extrapolated): 24 days (SFO)	radiochemical purity: > 99 % (HPLC)	Lewis, C.J. (2007) Report No.: 0333/257-D2149; QNM-0017
Photolysis Guideline: 95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 161-2 (1982), J MAFF Nousan-8147 section 2-6-2 (2000)	Fenpyrazamine, [pyrazolyl-^{14}C] and [phenyl-^{14}C] labels: DT_{50} values (also equivalent to natural summer sunlight in UK/US) of 1.7 days S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days).	radiochemical purity: ≥ 98 % (HPLC)	Lewis, C.J., Troth, K. (2007) Report No: 0333/258-D2149; QNM-0029
Biological degradation Guideline: Method C.4-C of Annex V of EU Directive 67/548/EEC, OECD 301 B	Not ready biodegradable	purity 94.7 %	Burwood, C.E., Scholey, A. (2006) Report No. 0333/261-D2149; QNM-0011
Water/Sediment Study Guideline: EC Directive 95/36/EC, OECD Guideline 308 (2002)	Water: DT50: 41 d DT90: 136 d Whole system: DT50: 68.1 d DT90: 226.3 d	radiochemical purity: > 99 % and > 98 % (HPLC)	Lewis, C.J., Troth, K. (2007) Report No: D2149-0333/260, QNM-0028 Jarvis, T., Callow, B., (2009) Report No: QNM-0040
Kinetic Evaluation of the Aerobic Aquatic metabolism (Menke, 2006c) Guideline: FOCUS Degradation Kinetics Report (FOCUS 2006)	Water: DT50: 25.5 d (geometric mean) DT90: 84.7 d (geometric mean) Whole system: DT50: 35.5 d (geometric mean) DT90: 117.9 d (geometric mean)		Lewis, C.J., Troth, K. (2007) Report No: D2149-0333/260, QNM-0028 Jarvis, T., Callow, B., (2009) Report No: QNM-0040

5.1.1 Stability

Hydrolysis:

Studies on the hydrolytic degradation were conducted with S-2188 at pH 4, 7 and 9, [phenyl-^{14}C] and [pyrazolyl-^{14}C] labels.

Reference:	**[14C]S-21988: Hydrolytic Stability**
Author(s), year:	Lewis, C.J., 2007
Study/report number:	0333/257-D2149; QNM-0017
Guideline(s):	EEC Method C.7 – Abiotic degradation. Hydrolysis as a function of pH (1992), OPPTS 835-2110 – Hydrolysis as a function of pH (1998), Japan MAFF New Test Guideline 12-Nousan 8147, section 2-6-1 (2001)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

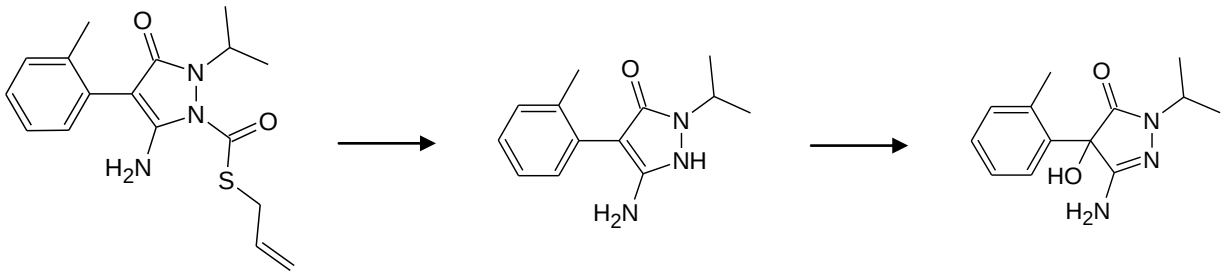
Test substance:	[Phenyl-14C]S-2188, 4.33 GBq mmol-1, > 99 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 2.04 GBq mmol-1, > 99 % radiochemical purity (HPLC), batch CFQ14368.
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI.
Test systems:	pH 4: 0.05 M citrate buffer (monopotatium citrate solution adjusted with sodium hydroxide) pH 7: 0.05 M phosphate buffer (potassium dihydrogen phosphate solution adjusted with sodium hydroxide) pH 9: 0.05 M borate buffer (sodium tetraborate solution adjusted with hydrochloric acid) All buffers sterilized by autoclaving. Oxygen content reduced by sonication and nitrogen bubbling.
Volatile traps:	No volatile traps (no volatiles expected – confirmed by complete material balance).
Test temperature:	Tier 1: 50 °C (pH 4 and pH 7), Tier 2: 25 °C (pH 9), 40 °C (pH 9) and 50 °C (pH 7 and pH 9), 60 °C (pH 7) and 70 °C (pH 7).
Test duration:	Up to 50 days in the dark.
Sample concentration:	1 mg L-1
Co-solvent:	Acetonitrile.
Analysis:	LSC, HPLC-UV/RAD, TLC LOQ < 1 % of AR (HPLC), < 0.1 % of AR (LSC).
Kinetic evaluation:	Simple first order (SFO) kinetics, Microsoft Excel.

Findings:

Mean material balances of all experiments were in a range of 93.9 – 101.7 % of AR. Owing to the complete mass balance $^{14}CO_2$ formation is considered to be negligible.

In Tier 1 test, S-2188 was hydrolytically stable at pH 4 and > 94 % of AR was recovered as the unchanged S-2188. At pH 7 more than 10 % hydrolysis occurred after 5-day application. Tier 1 test was not conducted at pH 9 because S-2188 is known to be unstable under alkaline conditions. The Tier 2 tests were subsequently conducted at pH 7 and pH 9. The product balances are presented in tables B.8.3.1-1 to B.8.3.1-3. Further incubations were undertaken at 60 and 70 °C (pH 7) and 40 and 50 °C (pH 9) but these were considered not to provide any further useful data and are not presented. No distinct differences between labels tested occurred.

Metabolite **S-2188-DC** was formed to a maximum occurrence of 59.4 % of AR (pH 7 at 50 °C, pyrazolyl label) and 49.0 % of AR for phenyl label at DAT 50. In trials at pH 9 and 25 °C metabolite S-2188-DC occurred at a maximum of 54.0 % of AR (pyrazolyl label) and 54.3 % of AR (phenyl label) at DAT 17. Metabolite **S-2188-OH** was found at a maximum occurrence of 10.0 % AR (pH 7 at 50 °C, phenyl label) and 7.4 % of AR with the pyrazolyl label at DAT 50. The maximum occurrence of S-2188-OH at pH 9 and 25 °C was 4.7 % of AR for the phenyl label and 5.1 % of AR for the pyrazolyl label at DAT 17. Unidentified radioactivity was below 10 % of AR.



S-2188 S-2188-DC S-2188-OH

Figure 1: Proposed hydrolysis degradation route of S-2188.

Conclusion:

S-2188 is stable at environmental relevant temperature at pH 4 and 7. S-2188 is rapidly degraded at alkaline pH of 9. The major hydrolysis product formed under sterile conditions and in the absence of air was S-2188-DC- This compound was almost hydrolytically stable but partially oxidised to S-2188-OH which never reached levels above 10 % of AR.

Comments (RMS):

None

Photolysis:

Studies on the photolytic degradation were conducted with S-2188 in sterile buffered water (pH 7.0), [pyrazolyl-^{14}C] and [phenyl-^{14}C] labels.

Quantum yield and half-life time under environmental conditions were determined for S-2188.

Reference:	**[14C]S-2188: Photodegradation and Quantum Yield in Sterile, Aqueous Solution.**
Author(s), year:	Lewis, C.J., Troth, K., 2007
Study/report number:	0333/258-D2149; QNM-0029
Guideline(s):	95/36/EC, 94/37/EC, SETAC (1995), US-EPA N 161-2 (1982), J MAFF Nousan-8147 section 2-6-2 (2000)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substances:	[Phenyl-14C]S-2188, 4.33 GBq mmol-1, ≥ 98 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 2.04 GBq mmol-1, ≥ 98 % radiochemical purity (HPLC), batch CFQ14368
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI, MPPZ
Test system:	Sterile pH 7.0 buffer (0.01 M phosphate buffer, autoclaved), adjusted with 2 M NaOH, sterility was checked throughout the experiment. A PNAP/PYR actinometer for determination of quantum yield.
Test temperature:	25 ± 1 °C
Test duration:	30 days continuous irradiation (1 day incubation equivalent to ca. 30 solar midsummer days in US and UK, ca. 3.3 days of natural Japanese spring sunlight) or dark incubation.
Sample concentration:	1.0 mg L^{-1}
Co-solvent:	Acetonitrile
Test system:	Xenon arc lamp (Suntest Accelerated Exposure machine), cut-off < 290 nm, ca. 25 watts m-2 (300 – 400 nm). Spectrum of experimental radiation is qualitatively similar to solar irradiation of Harrogate in summer. Three other Suntest machines were used and displayed similar radiation spectrum as the one presented.
Volatile traps:	Polyurethane bung, 1 x ethanediol trap and 2 x 2 M NaOH trap
Analysis:	LSC, HPLC-UV/RAD, TLC, HPLC-MS LOD < 0.1 % of AR (LSC), LOD < 0.5 % of AR (HPLC)
Kinetic evaluation:	Simple first order (SFO) kinetics, Microsoft Escel, ModelMker, curve fit based on mean values of both labels

Findings:

Mass balance was in a range of 94 to 100 % of AR for all experiments. Unit rinses contained ≤ 1.5 % of AR and hence confirmed no adsorption of radioactivity to the glass vessels. The ethanediol traps from the incubated samples contained no radioactivity during the entire incubation period whilst the polyurethane foam bungs contained only up to 1.4 % of AR. The

NaOH traps contained up to 1.5 % of AR from the [phenyl-^{14}C]S-2188 solution after 30 days and up to 10.3 % of AR from the [pyrazolyl-^{14}C] labelled S-2188 solution after 30 days (confirmed as CO_2 by barium hydroxide precipitation). Under irradiation S-2188 was subjected to extensive photolytic rearrangement procedures, resulting in two major metabolites: S-2188-DC (maximum 63.8 % of AR at DAT 7 for [pyrazolyl-^{14}C] label and 61.7 % of AR at DAT 7 for [phenyl-^{14}C] label) and MCNI (maximum 17.7 % of AR at DAT 30 for [pyrazolyl-^{14}C] label and 15.7 % of AR at DAT 30 for [phenyl-^{14}C] label). S-2188-DC degraded significantly until the end of the study. MCNI reached its maximum at the end of the study. Minor metabolites (S-2188-DTC and unk A) were detected but never reached levels above 10 % of AR. Unk A was characterised a dioxygenated compound of S-2188-DC. Without irradiation no degradation of S-2188 was observed and no metabolites were detected.

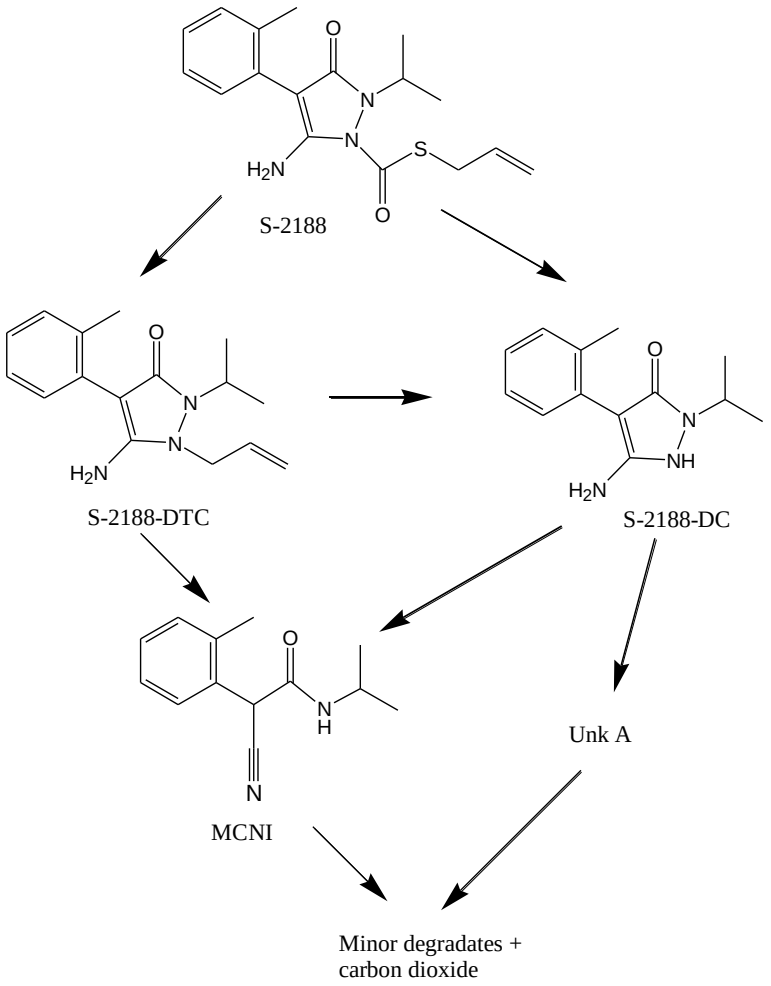


Figure 2: Proposed photolysis degradation route of S-2188

Table 10: Photo-transformation of S-2188 in sterile water buffered at pH 7 [% of AR]

Label	Conditions	DAT	S-2188	S-2188-DC	S-2188-DTC	MCNI	Unk A	Polar peak	Others	Undifferentiated/ unresolved	Total
[Pyrazolyl-^{14}C]	Irradiated	0	96.8	ND	ND	ND	ND	ND	ND	0.5	97.4
		1	63.4	29.7	1.2	ND	ND	ND	1.8	2.1	98.2
		2	38.5	47.7	2.1	0.4	2.1	ND	3.6	3.4	97.8
		3	29.5	55.3	2.2	0.6	1.3	ND	3.4	4.0	96.3
		7	7.1	63.8	4.2	2.6	3.8	ND	9.1	7.7	98.2
		20	2.0	48.1	4.4	9.0	2.8	4.2	10.7*	11.8	92.9
		30	1.1	9.5	4.2	17.7	5.0	13.9*	16.8*	18.7	86.8
	Dark	30	98.9	ND	ND	ND	ND	ND	ND	0.9	99.8
[Phenyl-^{14}C]	Irradiated	0	95.6	ND	ND	ND	ND	ND	ND	2.1	97.7
		1	62.3	23.7	1.1	0.3	2.0	ND	3.1	3.1	95.6
		2	40.8	36.4	2.1	1.0	3.0	ND	2.7	10.6	96.6
		3	26.5	54.7	3.0	1.0	3.8	ND	4.2	4.5	97.7
		7	4.4	61.7	4.8	4.0	3.5	ND	9.8	10.3	98.4
		20	1.0	37.1	4.7	9.9	8.5	3.4	15.9*	11.9	92.3
		30	1.6	7.4	6.3	15.7	4.7	4.6	30.1*	21.8	92.0
	Dark	30	93.2	ND	ND	ND	ND	ND	ND	3.1	96.4

* all individual peaks below 10 % of AR, ND: not detected

Table 11: Calculated aquatic photolytic DT50 and DT90 [days] of S-2188 and metabolites using SFO kinetics at pH 7

Compound	Label	DT_{50} (UK/US equivalent)	DT_{90} (UK/US equivalent)	r^2
S-2188	Pyrazolyl	1.7	5.5	0.985
	Phenyl	1.6	5.4	0.997
S-2188-DC	Pyrazolyl	13.2	43.9	n.s.
	Phenyl	11.8	39.3	n.s.
MCNI	Pyrazolyl	n.c.	n.c.	n.a.
	Phenyl	n.c.	n.c.	n.a.

n.s.: not stated; n.c.: not able to calculate; n.a.: not applicable.

Conclusion:

Under irradiated experimental conditions, S-2188 degraded with a half-life of 1.7 days at pH 7 and 25 °C (SFO kinetic) owing to photolysis. This experimental half-life corresponds to about 1.7 days under environmental conditions in US/UK summer (reference Harrogate 54 °N). Appropriate controls confirmed that there was no degradation in darkness. The main photolytic metabolites were S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days). A mean SFO DT_{50} value of 12.5 days was calculated for S-2188-DC. The degradation of MCNI could not be calculated as the maximum occurrence was reached at the end of the study period (DAT 30). Extensive breakdown of the molecule structure was observed after 30 days incubation as evidenced by the large number of minor unknown peaks and undifferentiated/unresolved radioactivity.

The quantum yield for S-2188 was determined to be 0.021.

Comments (RMS):

No comments. The study is considered acceptable.

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

Readily biodegradability:

Reference:	**S-2188: Assessment of Ready Biodegradability by Measurement of Carbon Dioxide evolution.**
Author(s), year:	Burwood, C.E., Scholey, A., 2006
Study/Report number:	0333/261-D2149; QNM-0011
Guideline(s):	Method C.4-C of Annex V of EU Directive 67/548/EEC, OECD 301 B
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	S-2188 (unlabelled), purity 94.7 %, batch 030-050914-1G
Reference substance:	Sodium benzoate
Inoculum:	Aeration tank of a waste water plant (Burley Menston) treating predominately domestic sewage (30 mg L-1)
Treatments:	Replicates (except for toxicity control):

	Blank control Reference substance: Na-benzoate (3 mL mg L-1) Test substance: S-2188 (3 mL L-1) Toxicity control: S-2188 (3 mL L-1) and Na-benzoate (3 mL mg L-1) pH of the test vessel at the end of test: 7.5 to 7.7
Analysis:	CO2 amount absorbed by each trap calculated from the reduction in the concentration of barium hydroxide solution (titration)
Incubation conditions:	21 ± 1 °C, 28 days

Findings:

Table 12: Biodegradation of S-2188 and reference compound [% of theoretically possible degradation]

DAT	Reference substance		Test substance	Toxicity control
	Replicate 1	Replicate 2		
2	34	10	0	29
3	45	19	0	39
6	56	30	0	51
8	62	37	0	56
10	66	47	0	59
13	70	44	0	61
14	72	47	0	63
17	75	50	0	65
20	78	54	0	69
24	83	58	0	73
28	90	64	1	79

Within 28 days almost no degradation (maximum 1 %) was determined for S-2188. The reference substance (Na-benzoate) has reached level for ready biodegradability by 8 and 28 days.

Conclusion:

S-2188 is considered to be not readily biodegradable.

Comments (RMS):

None

5.1.2.3 Simulation tests

Biodegradation in water/sediment systems:

One water sediment study was conducted:

- Aerobic water/sediment study with two test systems, [phenyl-^{14}C] and [pyrazolyl-^{14}C] label

Reference:	[14C]S-2188: Degradation and Retention in Water-Sediment Systems
Author(s), year:	Lewis, C.J., Troth, K., 2007
Study/report number:	D2149-0333/260, QNM-0028
Guideline(s):	EC Directive 95/36/EC, OECD Guideline 308 (2002)
GLP:	Yes
Deviations:	Minor deviations
Validity:	Study considered acceptable

Reference:	Determination of rates of degradation for S-2188 from a water sediment study incubated under laboratory conditions.
Author(s), year:	Jarvis, T., Callow, B., 2009
Report/Doc. number:	QNM-0040
Guideline(s):	FOCUS Degradation Kinetics Report (FOCUS 2006)
GLP:	Not applicable
Deviations:	None
Validity:	Yes

Material and methods:

Test substances:	[Phenyl-14C]S-2188,12.9 MBq mg-1, > 98 % radiochemical purity (HPLC), batch CFQ14367 [Pyrazolyl-14C]S-2188, 6.1 MBq mg-1, > 99 % radiochemical purity (HPLC), batch CFQ14368
Reference substances:	S-2188 (unlabelled), S-2188-OH, S-2188-DC, S-2188-DTC, MCNI
Application rate:	0.08 µg mL-1 (under the assumption of 750 g ai ha-1, 100 cm water depth)
Co-solvent:	Acetonitrile (0.005 %, v/v)
Incubation set-up:	3 cm depth of dry Calwich Abbey or Swiss Lake sediment (2 mm sieved) in individual borosilate glass cylinder (ca 4.5 cm in diameter), 9 cm depth of associated water (0.2 mm sieved). Sediment units were aerated, slightly agitated on an orbital shaker. Flow through system (moistured air at flow rate of ca. 20-60 mL min-1).
Acclimatization period:	Approx. 35 days
Test duration:	100 days
Incubation conditions:	20 ± 2 °C in darkness
Volatile traps:	Ethanediol (polar volatiles), 2 % paraffin in xylene (non-polar volatiles), sodium hydroxide (CO2), addition of barium chloride slution to confirm presence of 14CO2.
Analysis:	Water phase mixed with 130 mL acetonitrile and radio-assayed by LSC and analysed by chromatography. Sub-samples (ca 10 mL) collected and added to 2 M sodium hydroxide solution to determine presence of

	dissolved CO2. Sediment phase extracted 4 times with 100 mL of methanol:water (5:1 v/v, neutral extraction) and 3 times with 100 mL methanol:0.5 M hydrochloric acid (5:1 v/v, acidic extraction). Each extract type was combined and aliquots (200 µL) were analysed by LSC. Remaining sediment residues were dried and ground for combustion. 100-day sample: further extraction performed. One replicate was Soxhlet extracted for ca. 16 hours with 100 mL acetone:0.5 M hydrochloric acid (5:1 v/v), analysed by LSC. Non extractable residues: were separated into humic acids, fulvic acids and humins. Radioactivity from fulvic acid and humic acid was determined by LSC. Radioactivity from humin fraction was determined by combustion followed by LSC. Trapping solutions: radioactivity quantified by LSC.
Analytical techniques:	LSC (LOD ca 0.1 % of AR), HPLC-UV/RAD (LOD ca 0.5 % of AR), TLC, LC-MS (API)
Kinetic evaluation:	Simple first order (SFO) kinetics, first order multi-compartment (FOMC), KinGui v. 1.1 (BCS 2006), ModelMaker 4, FOCUS_DEGKIN v2.xls

Table 13: Physicochemical characteristics of the water/sediment matrices.

Name		**Calwich Abbey**	**Swiss Lake**
Geographic location		**Calwich Abbey lake, Calwich, Ashbourne, Derbyshire, UK**	**Swiss Lake, Chatsworth, Derbyshire, UK**
Sediment	Texture (USDA)	Silty clay loam or Clay loam	Sand
	Sand (USDA) [%]	41	91
	Silt (USDA) [%]	25	7
	Clay (USDA) [%]	34	2
	pH (1 M KCl)	7.3	6.0
	pH (water)	7.5	6.1
	Organic C [%]	4.6	0.6
	Redox [mV]	-236	-230
	CEC [mEq 100 g^{-1}]	19.8	5.4
	Microbial Biomass [µg C g^{-1}] – Start / End	1052 / 1666	157 / 197
Water	pH (sampling)	8.6	6.4
	Water hardness [mg L^{-1} as $CaCO_3$] – Start / End	145 / 149	57 / 52
	Oxygen content [mg L^{-1}]	7	8
	Conductivity [µS cm^{-1}]	99	92
	Redox [mV] – Start	160	178
	TOC [ppm] – Start / End	74 / 16	77 / 18
	Suspended solids [mg L^{-1}] – Start / End	25 / 42	2 / 63

Findings:

The oxygen content of the water phase of the samples ranged mostly between 7 and 10 mg L^{-1} in both systems. This indicates aerobic conditions throughout both experiments. The pH in the water phase decreased from pH 8.2 - 8.3 at the start of the study to pH 7.6 - 8.1 at the end in the Calwich Abbey water sediment system and from pH 7.3 - 8.0 at the start to pH 4.1 -5.6 at the end

of the study in the Swiss lake system. The pH of the sediment of Calwich Abbey rose slightly from 0 DAT (pH 6.7 - 7.0) to pH 7.4 at the end of the study. In the Swiss Lake system the pH in sediment decreased from pH 6.5 at the start to pH 4.6 - 5.7 at 100 DAT. The water redox potential evolved from values between +92 and +123 mV (Calwich Abbey) and between +88 and +102 mV (Swiss Lake) at 0 DAT to final values between +143 and +227 mV (Calwich Abbey) and between +256 and +411 mV (Swiss Lake). In the sediment of Calwich Abbey the redox potential was in the range of -130 and -155 mV at 0 DAT and between -70 and -128 mV at 100 DAT. In the sediment of Swiss Lake the redox potential increased from values between -118 and -125 mV at the start of the study to values between +37 and +90 mV at the end of the study.

Total mass balance was in a range of 92.2 to 100.0 % of AR for both systems and both labels. Formation of $^{14}CO_2$ using [pyrazolyl-^{14}C] label accounted for maximum 8.5 % of AR in the Calwich Abbey system and for maximum 3.3 % of AR in the Swiss lake system at study termination. Formation of $^{14}CO_2$ using [phenyl-^{14}C] label accounted for maximum 5.5 % of AR in the Calwich Abbey system and 3.1 % of AR in the Swiss lake system. Formation of NER increased up to maximum 47 % of AR with [pyrazolyl-^{14}C] label and to 47.4 % of AR with the [phenyl-^{14}C] label in the Calwich Abbey system, and to 19.5 % of AR [pyrazolyl-^{14}C] label and to 17.2 % of AR with the [phenyl-^{14}C] label in the Swiss lake system.

Distribution and recovery of radioactivity in both water/sediment systems are presented in tables B.8.3.4.3-2 and B.8.3.4.3-3. Only data on major fractions are shown. Therefore, the mass balance values presented in the tables below do not fit the presented data. In all incubations there was no radioactivity in the traps for volatile organic compounds. MCNI reached a maximum of 0.7 % of AR in sediment in the Calwich Abbey system and was never detected in the water. One unknown compound, Unk E (postulated MW=172.16 g mol^{-1}) reached a maximum of 7.4 % of AR in water and 1.7 % of AR in sediment (9.1 % of AR in the total system). Up to six other unidentified metabolites were quantified but all were below 10%.

Table 14: Distribution and recovery of radioactivity [% of AR] after application of [pyrazolyl-14C] labelled S-2188 (750 g ai ha-1) to the aerobic water/sediment systems 'Calwich abbey' and 'Swiss lake'.

System	Time	Water			Sediment			Unextractable	CO_2	Mass balance	Total water/sediment		
	(day)	S-2188	S-2188-DC	S-2188-OH	S-2188	S-2188-DC	S-2188-OH				S-2188	S-2188-DC	S-2188-OH
Calwich abbey	0	90.5	0.8	ND	NA	NA	NA	0.1	NA	98.7	90.5	0.8	0.0
	7	46.7	10.2	2.4	24.7	6.1	0.9	2.3	0.5	98.1	52.8	11.1	4.7
	14	38.4	3.7	7.0	19.7	6.1	1.1	7.9	1.3	92.2	44.5	4.8	14.9
	30	7.8	2.6	6.8	18.7	6.3	3.8	24.2	2.5	94.9	14.1	6.4	31.0
	61	1.6	4.3	12.2	4.1	4.4	3.7	42.3	3.9	93.6	6.0	8.0	54.5
	100	3.1	3.8	6.1	2.0	3.1	4.4	47.0	8.5	95.7	6.2	8.2	53.1
Swiss lake	0	94.5	1.5	ND	NA	NA	NA	ND	NA	100.0	94.5	1.5	0.0
	7	53.1	2.6	2.5	30.3	0.3	0.2	0.8	0.7	96.3	53.4	2.8	3.3
	14	41.4	1.4	5.0	32.8	0.7	0.8	2.6	1.4	97.0	42.1	2.2	7.6
	30	34.2	1.5	9.1	23.0	2.5	2.5	7.1	1.4	97.7	36.7	4.0	16.2
	61	21.4	0.6	8.2	22.2	1.2	1.9	13.3	3.1	93.2	22.6	2.5	21.5
	100	13.5	0.9	7.3	27.6	3.2	3.2	19.5	3.3	98.6	16.7	4.1	26.8

Table 15: Distribution and recovery of radioactivity [% of AR] after application of [phenyl-14C] labelled S-2188 (750 g ai ha-1) to the aerobic water/sediment systems 'Calwich abbey' and 'Swiss lake'.

System	Time (day)	Water			Sediment			Unextractable	CO_2	Mass balance	Total water/sediment		
		S-2188	S-2188-DC	S-2188-OH	S-2188	S-2188-DC	S-2188-OH				S-2188	S-2188-DC	S-2188-OH
Calwich abbey	0	93.6	0.4	0.3	NA	NA	NA	ND	NA	99.6	93.6	0.4	0.3
	7	48.3	11.9	2.6	21.4	8.6	1.0	1.3	0.3	98.1	69.7	20.5	3.6
	14	40.7	2.6	5.8	20.0	10.5	1.8	7.0	0.7	95.2	60.7	13.1	7.6
	19	24.3	2.8	7.2	24.2	7.8	1.8	12.4	1.4	98.2	48.5	10.6	9.0
	30	14.5	2.4	7.3	17.1	6.6	2.8	19.3	1.7	93.3	31.6	9.0	10.1
	61	2.2	3.0	7.8	9.4	3.7	2.6	37.6	4.4	95.6	11.6	6.7	10.4
	100	0.3	5.5	9.2	2.1	3.8	3.6	47.4	5.5	93.1	2.4	9.3	12.8
Swiss lake	0	95.8	0.7	0.1	NA	NA	NA	ND	NA	99.4	95.8	0.7	0.1
	7	67.0	0.8	3.0	22.4	0.5	0.2	0.6	0.4	98.1	89.4	1.3	3.2
	14	54.0	1.3	5.4	29.2	0.9	0.6	1.3	0.6	99.4	83.2	2.2	6.0
	30	39.9	1.4	12.5	20.1	1.7	1.8	4.6	1.9	97.5	60.0	3.1	14.3
	61	18.6	0.7	10.3	29.0	0.3	3.0	16.4	2.9	99.4	47.6	1.0	13.3
	100	11.8	1.5	9.3	25.9	2.5	2.6	17.2	3.1	96.8	37.7	4.0	11.9

S-2188 degraded in both water/sediment systems with a faster degradation rate in the Calwich Abbey system than in the Swiss Lake system. Transfer of the test substance into sediment was relatively fast with occurrences of S-2188 between 21.4 and 30.3 % of AR after 7 days. Maximum occurrence of [pyrazolyl-^{14}C] labelled S-2188 in sediment was 24.7 (DAT 7) and 32.8 % of AR (DAT 14) in Calwich Abbey and Swiss Lake respectively. For [phenyl-^{14}C] labelled S-2188 the maximum occurrence in sediment was 24.2 % of AR (DAT 19) and 29.2 % of AR (DAT 14) in Calwich Abbey and Swiss Lake respectively. In the Calwich Abbey system the degradation of S-2188 led to the formation of the major metabolite **S-2188-DC** at concentrations in water between 10.2 (DAT 7) and 11.9 % (DAT 7) of AR with the [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelling respectively. In sediment the metabolite reached maximum levels of 6.3 % of AR (DAT 30) for the [pyrazolyl-^{14}C] label and 10.5 % of AR (DAT 14) for the [phenyl-^{14}C] label. Metabolite S-2188-DC did not reach levels above 10 % of AR in the Swiss Lake neither in water nor in the sediment phase. Metabolite S-2188-DC further degraded into metabolite **S-2188-OH** which reached 12.2 % of AR ([pyrazolyl-^{14}C] label) after 61 days in the water phase of the Calwich Abbey system and 12.5 % of AR ([phenyl-^{14}C] label) after 30 days in the water phase of the Swiss Lake system. S-2188-OH was not detected at concentrations above 5 % of AR in sediment in both systems with both labelling.

The proposed degradation pathway of S-2188 in water/sediment system is shown in the figure below. The degradation rates were fitted with Single First Order (SFO) kinetic. For comparative purposes First Order Multi-Compartment (FOMC) kinetic was also presented. SFO kinetics was considered acceptable for determining simulation endpoints allowing good visual fit and χ^2 error values below 8 %. Too few data values were available to calculate degradation rates of metabolites S-2188-DC and S-2188-OH.

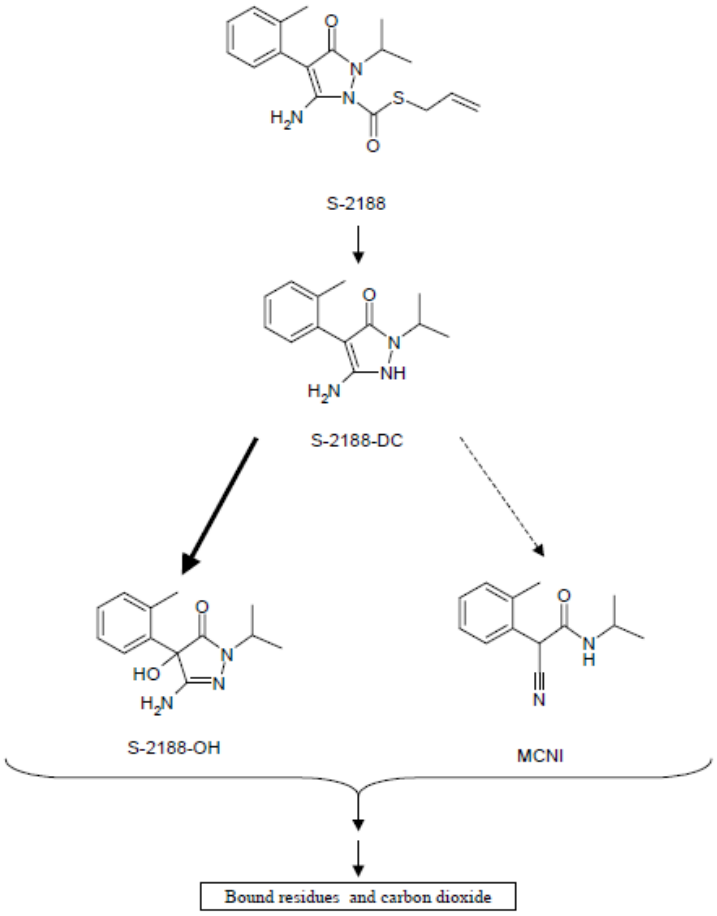


Figure 3: Proposed metabolic pathway for S-2188 in water-sediment systems

Table 16: Degradation rates of S-2188 in water sediment systems (whole system) following Single First Order (SFO) kinetics and First Order Multi-Compartment (FOMC) kinetics for comparison purposes.

Parameter	Calwich Abbey		Swiss Lake	
	[Pyrazolyl-^{14}C]	[Phenyl-^{14}C]	[Pyrazolyl-^{14}C]	[Phenyl-^{14}C]
Model	SFO	SFO	SFO	SFO
Chi^2 error [%]	5.3	3.4	7.1	5.0
k [day^{-1}] *	0.0385 (1.3 x 10^{-4})	0.0345 (2.2 x 10^{-6})	0.0102 (0.0026)	0.0108 (6.2 x 10^{-4})
DT_{50} [Day]	18.0	20.1	68.1	64.4
DT_{90} [Day]	59.9	66.7	226.3	214.0

*P value from the t-test is given in brackets.

The geometric mean DT_{50} for S-2188 in the water sediment system (whole system) was calculated to 35.5 days. Half-lives of S-2188 in the water phase and in the sediment phase of the water sediment systems are presented in following table .

Table 17: Calculated half-lives [days] of S-2188 in the water and sediment phase in the aerobic water/sediment systems Calwich Abbey and Swiss Lake based on SFO kinetics.

System	Label	Water			Sediment			Kinetics
		Dissipation			Dissipation			
		DT_{50} [days]	X^2 error [%]	T-test [p value]	DT_{50} [days]	X^2 error [%]	T-test [p value]	
Calwich Abbey	[Pyrazolyl-^{14}C]	28.8	11.3	0.141	11.5	17.1	0.071	SFO
Calwich Abbey	[Phenyl-^{14}C]	19.8	9.4	0.012	21.3	10.6	0.089	SFO
Swiss Lake	[Pyrazolyl-^{14}C]	18.0	5.3	0.062	n.c.	17.4	n.c.	SFO
Swiss Lake	[Phenyl-^{14}C]	41.0	5.8	0.093	363	20.9	0.449	SFO

n.c.: not calculable, k value negative.

Conclusion:

S-2188 degraded via S-2188-DC and S-2188-OH, with both metabolites exceeding 10% in the water phase and the overall system (maximum in whole system 20.5 % of AR and 54.5 % of AR, respectively). In addition, S-2188-DC exceeded 10% in the sediment phase alone. No other compounds were detected at levels above 10 % of AR in the overall system. Mineralisation over 100 days was relatively small (3.1 - 8.5 % of AR) and not greatly different between the [phenyl-^{14}C] and [pyrazolyl-^{14}C] labelled forms of S-2188. Amounts of bound residues remaining after 100 days were also very similar between the two radiolabelled forms, but different between the two water/sediment systems being about 47 % of AR for Calwich Abbey (high organic carbon) and about 18 % of AR for Swiss Lake (low organic carbon). S-2188 dissipated from the water and reached maximum amounts of 32.8 % of AR in the sediment phase (DAT 14). Degradation in the whole system was determined using SFO (following guidance document FOCUS, 2006) with first order DT_{50} values of 18.0 – 68.1 days (geomean 35.5 days) and DT_{90} values of 59.9 - 226.3 days.

Comments (RMS):

The study is considered correct.

5.1.3 Summary and discussion of degradation

Aquatic hydrolysis

One hydrolysis study was carried out in dark sterile buffer solutions at pH 4, 7 and 9 at 50 °C for pH 4 and pH 7 (Tier 1), 25 °C and 40 °C (pH 9), 50 °C for pH 7 and pH 9, 60 °C and 70 °C for pH 7 using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled S-2188. S-2188 was hydrolytically stable at environmental temperature at pH 4 and 7 but at pH 9, degradation to S-2188-DC and subsequently to S-2188-OH occurred. At 20 ºC and pH 9 the DT_{50} for hydrolysis was 24 days. As the degradation rate of fenpyrazamine by hydrolysis at 20 °C takes longer than 16 days the active substance can not be considered to be rapidly hydrolysed.

Aquatic photolysis

Aquatic photolysis of S-2188 was investigated in sterile buffer solutions at pH 7.0 using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled parent. The test systems were continuously irradiated with a xenon arc lamp (> 290 nm) for 30 at 25 °C to simulate the impact of natural light. Under irradiation in sterile buffer solutions at pH 7.0, S-2188 degraded with a half-life of 1.7 days (following SFO kinetics). The experimental half-life corresponds to 1.7 days under environmental summer sunlight in UK/US. Appropriate controls confirmed that there was no degradation in

darkness. The main photolytic products were S-2188-DC (maximum occurrence of 63.8 % of AR at DAT 7) and MCNI (maximum occurrence of 17.7 of AR at DAT 30). A mean SFO DT_{50} value of 12.5 days was calculated for S-2188-DC. MCNI reached its maximum at the end of the study and therefore no degradation rate could be calculated. Extensive breakdown of the molecule structure was observed after 30 days incubation as evidenced by the large number of minor unknown peaks and undifferentiated/unresolved radioactivity. The quantum yield for S-2188 was determined to be 0.021.

Biological degradation

Results of a **readily biodegradability study** indicate that S-2188 is not readily biodegradable.

Dark aerobic **water/sediment studies** were conducted with two contrasting (pH, texture) natural systems, Calwich Abbey and Swiss Lake, using [pyrazolyl-^{14}C] and [phenyl-^{14}C] labelled S-2188. The Calwich Abbey test system represents a silty clay loam or clay loam sediment with an organic carbon content of 4.6 %, a microbial biomass of 1052 µg C g^{-1} and a pH of 7.3 (KCl). The Swiss Lake test system is characterized by a sand sediment with a pH of 6.0 (KCl), with an organic carbon content of 0.6 % and a lower microbial biomass (157 µg C g^{-1}). In both systems, water and sediment stayed aerobically throughout the test period.

Mineralisation of S-2188 using [phenyl-^{14}C] label accounted by study termination for maximum 5.5 % of AR in the Calwich Abbey system and 3.1 % of AR in the Swiss lake system. Formation of NER increased up to maximum 47 % of AR with [pyrazolyl-^{14}C] label and to 47.4 % of AR with the [phenyl-^{14}C] label in the Calwich Abbey system, and to 19.5 % of AR [pyrazolyl-^{14}C] label and to 17.2 % of AR with the [phenyl-^{14}C] label in the Swiss lake system.

In the total system, decline of S-2188 was observed with $DegT_{50}$ of 19 and 66 days in the Calwich Abbey respectively the Swiss Lake test system (following SFO kinetics), respective $DegT_{90}$ values were 63 and 220 days. Geomean was calculated to 35.5 days. Dissipation in the water phase was calculated to be 24 and 30 in the Calwich Abbey and Swiss Lake test systems respectively. No distinct differences between labels used were observed.

Major metabolites S-2188-DC and S-2188-OH both exceeded 10% in the water phase and the overall system (maximum in whole system 20.5 % of AR and 15.9 % of AR, respectively). In addition, S-2188-DC exceeded 10% in the sediment phase alone. No degradation rates were calculated for the major metabolites S-2188-DC and S-2188-OH.

Table 18: Summary on DT50 and DT90 [days] for the dissipation and degradation of S-2188, S-2188-DC and S-2188-OH in aerobic water/sediment studies.

Compound	Test system	Water				Sediment		Total system	
		Degradation		Dissipation		Dissipation		Degradation	
		$DegT_{50}$	$DegT_{90}$	DT_{50}	DT_{90}	$DegT_{50}$	$DegT_{90}$	$DegT_{50}$	$DegT_{90}$
S-2188	Calwich Abbey	nc	nc	23.9	79.3	15.7	52.1	19.0	63.2
	Swiss Lake	nc	nc	27.2	90.3	nc	nc	66.2	220.1
	Geometric mean	nc	nc	25.5	84.7	nc	nc	35.5	117.9
S-2188-DC	Calwich Abbey	nc	nc	nc	nc	nc	nc	nc	nc
	Swiss Lake	nc	nc	nc	nc	nc	nc	nc	nc
	Geometric mean	nc	nc	nc	nc	nc	nc	nc	nc
S-2188-OH	Calwich Abbey	nc	nc	nc	nc	nc	nc	nc	nc
	Swiss Lake	nc	nc	nc	nc	nc	nc	nc	nc
	Geometric mean	nc	nc	nc	nc	nc	nc	nc	nc

nc denotes not calculated

Table 19: Summary on maximum occurrence [% of AR] of S-2188 and metabolites in aerobic water/sediment studies (mean of labels used, data stated in brackets give day of maximum occurrence).

Compound	Water	Sediment	Total
S-2188	-	31.0 (14)	-
S-2188-DC	11.1 (7)	8.3 (14)	15.8 (7)
S-2188-OH	10.8 (30)	4.0 (100)	32.5 (61)

The criteria for rapid biological degradation are not met, as the substance is not ready biodegradable and the whole system DegT50 is greater than 16 days.

5.2 Environmental distribution

Route of degradation in soil

The route of degradation of the active substance fenpyrazamine (S-2188) was established on 4 EU soils in one study S-2188. The aerobic laboratory soil degradation study was conducted under flow-through conditions (moistured air at a flow rate of ca 20-60 mL min^{-1}). Under standard **aerobic conditions** (20 °C), S-2188 ([phenyl-^{14}C] and [pyrazolyl-^{14}C] labels) degraded to CO_2 and bound residues. No volatile organics could be detected. The CO_2 concentrations were in the range of 5.2 to 8.5 % of AR at the end of the study (120 DAT). The NER concentrations increased steadily during the study period to reach at the end of the study values between 38.9 and 69.9 % of AR. Two metabolites were identified, S-2188-DC and S-2188-OH, but were always < 5 % of AR in the four soils. No **anaerobic degradation** study was presented as the substance is to be applied to grapes during the summer months (in North and South Europe) and to fruiting vebgetables under glasshouse conditions. S-2188 is considered to be stable to **photolysis** on soil.

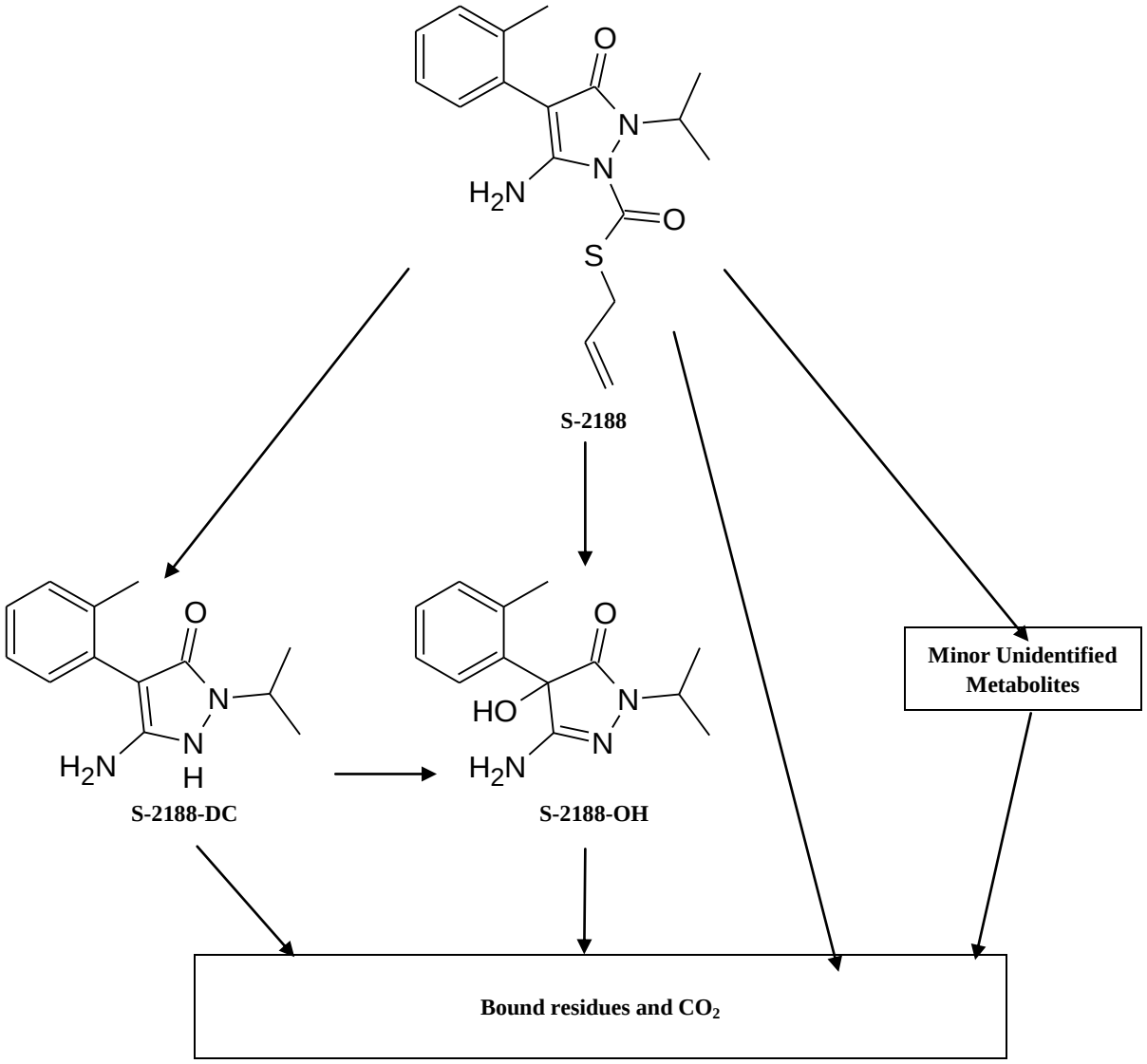


Figure 4: Proposed metabolic pathway for fenpyrazamine (S-2188) in soil

Rate of degradation in laboratory studies

The laboratory soil degradation rate of S-2188 was investigated in 4 EU soils with the following range of soil properties (pH, organic C, clay content) using [phenyl-^{14}C] and [pyrazolyl-^{14}C] labelled S-2188 as parent.

- pH ($CaCl_2$) 4.3 – 6.8
- organic C 1.7 – 4.2 %
- clay content 10 – 26 %

Under **aerobic conditions**, S-2188 was found to degrade following biphasic degradation in two soils and SFO kinetic in two other soils. Following best fit kinetics in soils PT102 and SK920191 (i.e. single first order, SFO) non-normalised DT_{50} values were between 23.6 and 40.0 days (pyrazolyl-label) and 33.5 days for the phenyl label. In soils PT103 and SK15556090 following the best fit kinetics (i.e. double first order in parallel kinetic, DFOP), non-normalized degradation

half-life ($DegT_{50}$) values of S-2188 were 39.0 and 28.7 days respectively (chi^2 error ≤ 3.0 %); respective $DegT_{90}$ values were 1846.8 and 515.5 days. Since most environmental models are not capable to handle DFOP kinetics, a conservative SFO-$DegT_{50}$ for modelling may be derived from the DFOP-slow rate k values. This procedure results in non-normalised $DegT_{50recalc}$ values of 888.0 and 230.1 days respectively. These values are extrapolated past the incubation time.

Two aerobic soil metabolites were identified (S-2188-DC and S-2188-OH) but did not reach values above 5 % of applied radioactivity.

No rate of degradation for **photolysis** on soil could be calculated since S-2188 is stable to photolysis.

Field dissipation studies

One field dissipation study (bare soils) was conducted with S-2188 on four European soils. The substance was applied at 1200 g a.s. ha^{-1} (single application) as a 50 % WG formulation.

Dissipation of S-2188 in field trials was following multi-compartment kinetics and dissipation half-lifes (DT_{50}) ranged between 7.7 and 39.8 days (pseudo-DT_{50} from FOMC kinetics or slow phase DT_{50} from DFOP kinetics). Since almost no transfer of S-2188 into soil layers below 10 cm was observed and volatilization is considered to be minimal (vapour pressure < 10^{-5} Pa), dissipation of S-2188 is considered more or less consistent with degradation.

No metabolites were observed in the field trials.

Table 20: Summary of the results of the kinetic determinations for S-2188 at the field dissipation sites following normalisation of data to 20°C and pF2.

	UK site (Site 0333/266/1)	**German site (Site 0333/266/2)**	**Italian site (Site 0333/266/3)**	**French site (Site 0333/266/4)**
Model	SFO	SFO	SFO	SFO
χ^2 error [%]	24.2	29.1	40.2	28.2
k [day^{-1}] *	0.156	0.5082	0.0985	0.0547
	(0.0019)	(0.0035)	(0.0129)	(0.0046)
DT50 [day]	4.4	1.4	7.0	12.7
DT90 [day]	14.8	4.5	23.4	42.1
Model	FOMC	FOMC	FOMC	FOMC
χ2 error [%]	11.4	10.0	20.0	19.9
α*	0.6249	0.5526	0.4855	0.7555
	(0.0011)	($6.7x\ 10^{-5}$)	(0.0045)	(0.0093)
β*	1.7278	0.4030	0.9022	4.197
	(0.0344)	(0.0117)	(0.1378)	(0.0959)
DT_{50} [day]	3.5	1.0	2.9	6.3
DT_{90} [day]	67.1	25.6	102.6	84.2
Model	DFOP	DFOP	DFOP	DFOP
χ^2 error [%]	Not required	Not required	8.1	10.0
k fast*	-	-	1.7297	3.0350
	-	-	(0.4330)	(0.4990)
k slow*	-	-	0.0174 (2.2×10^{-4})	0.0243 (5.8×10^{-4})
g*	-	-	0.5800 (1.2×10^{-6})	0.4339 (1.2×10^{-4})
DT_{50} [day]	-	-	1.1	5.1
DT_{90} [day]	-	-	82.4	71.3

*P value from the t-test is given in brackets. Selected kinetic fit for each soil highlighted in light grey.

5.2.1 Adsorption/Desorption

Reliable adsorption constants according to Freundlich isotherms (equilibrium batch experiments) could be achieved for **S-2188** using 5 soils from the EU and Japan with a representative set of soil properties:

- Soil pH ($CaCl_2$): 4.2 – 7.5
- Organic carbon: 0.8 – 4.8 %
- Clay: 9 – 28 %

No dependency of the adsorption behaviour onto the soil pH could be stated in the pH range tested. Freundlich adsorption constants (K_F) of S-2188 have been determined in batch equilibrium experiments with five different soils using the [phenyl-^{14}C] labelled test substance. Based on organic carbon content, K_{FOC} values for the different soils were in a range of 112 – 731 L kg^{-1} (arithmetic mean 1/n = 0.911). Based on these values, S-2188 is classified as medium mobile according to the classification scheme of McCall et al. (1981).

Table 21: Adsorption/desorption characteristics of [14C]S-2188 on five soils

Soil	Organic carbon [%]	pH ($CaCl_2$)	Adsorption			Desorption		
			K_F^{ads}	K_{Foc}	1/n	K_F^{des}	$K_{Foc-des}$	1/n
SK961089	4.8	7.5	9.36	195	0.8801	10.82	225	0.8592
SK104691	2.7	6.1	7.87	292	0.9055	9.11	338	0.8918
SK179618	3.8	5.5	4.27	112	0.9321	5.07	133	0.9293
SK566696	0.8	4.2	5.85	731	0.9525	7.63	954	0.9507
Saitama	3.2	5.3	6.99	218	0.8855	8.62	269	0.8950
Mean (n=5)				310	0.9111		384	0.9052

5.2.2 Summary of behaviour in soil

The route of degradation of S-2188 was studied in four soils (pH 4.3 - 7.4, %OC 1.7 - 4.2, sandy loam to clay loam) under aerobic conditions using compound radiolabelled ([phenyl-^{14}C] or [pyrazolyl-^{14}C] rings). Levels of CO_2 after 120 days incubation were similar for either radiolabelling position, being 5.2 - 8.5 % of AR. Levels of bound residues showed greater variation between soils types than between labelling positions and were 38.9-69.9%, at the completion of the 120 day incubation. Two metabolites, S-2188-OH and S-2188-DC were identified but did not reach levels above 5 % of AR in any soil. There was no evidence of any cleavage between the phenyl and pyrazolyl rings. A proposed degradation pathway is shown in Figure B.8.1.1-2. Soil photolysis studies showed greater degradation under non-irradiated conditions than irradiated conditions (and the same metabolites were present as in the aerobic soil degradation study), hence it was concluded that S-2188 is stable to photolysis. S-2188 will be applied to grapes in North and South Europe during the summer months, and to fruiting vegetables under glasshouse conditions. Therefore it is unlikely to be present in soil during waterlogged (anaerobic) conditions in winter and hence an anaerobic soil degradation study was not considered relevant.

The rate of degradation in four soils under laboratory conditions was studied. In two soils degradation was clearly seen to follow SFO kinetics and DT_{50} values of 23.6 - 39.9 days were obtained. In the other two soils, a more biphasic degradation was seen to follow DFOP kinetics and DT_{50} values of 28.8 – 39.2 days were obtained (although the chi^2 error was still acceptable for SFO kinetics). Following the FOCUS kinetics group guidance, the best fit kinetics was used as persistence endpoints to trigger further studies. Hence DT_{50} values were 23.6 - 39.9 days and DT_{90} values were 78.3 - >1000 days. Based on these values a field dissipation study is not

triggered since DT_{50} is below 60 days. However, a study was undertaken at four sites (UK, Germany, Italy, Southern France) and the data were normalised following the time step normalisation. Best fit for the UK and German sites were obtained with FOMC kinetic, and for the Italian and French sites with DFOP kinetic. The range of pseudo-DT_{50} (FOMC kinetic) and DT_{50} values calculated from the slow rate k value (DFOP kinetic) ranged between 7.7 and 39.8 days.

For use as simulation input values, the FOCUS (2006) document recommends in the first instance using the slow phase from the DFOP kinetics as a first order value to be input into the models if the fit to SFO kinetics is not acceptable. However, this leads to unrealistic degradation rates in the PT103 and SK15556090 soils (equivalent SFO DT_{50} values would be 231-936 days based on the slow rate from DFOP kinetics) since the DFOP fit is clearly strongly influenced by minor variation at the later timepoints. For simulation purposes the Notifier proposed to use SFO kinetics data for PT103 and SK15556090 soils. The values obtained (DT_{50} 67.9 and 56.4 days, respectively (DT_{90}s 225.7 and 187.5 days)) were consistent with the other two laboratory degradation studies and the field dissipation study. The use of SFO was not considered adequate by RMS since even if the χ^2 error values were below 15 % the visual fit was not satisfactory as supported by the biased residual plots. The consistency to the other two degradation studies and the field study was not considered as a valid argument. Therefore the field degradation studies were normalised and the obtained geometric mean DT_{50} value of 20.5 days was used for modelling purposes.

The sorption of S-2188 was studied in four European soils and one Japanese soil (pH range 4.2 to 7.5, % OC 0.8 – 4.8 loamy sand to clay loam). Adsorption K_{Foc} values were between 112 and 731 ml g^{-1} (mean 310 ml g^{-1}, mean 1/n = 0.911) and desorption $K_{Foc\text{-}des}$ values were between 133 and 954 ml g^{-1} (mean 384 ml/g, mean 1/n= 0.905) indicating that S-2188 is slightly to moderately mobile. There was no evidence of any pH dependence. No metabolites reached levels above 3 % of AR in soil and hence there was no need for further sorption or column leaching studies.

5.2.3 Volatilisation

S-2188 has a very low vapour pressure of below 10^{-5} Pa at 25 ºC (see Annex IIA point 2.3.1) and is predicted to degrade rapidly in air through reaction with hydroxyl radicals (DT_{50} of 1.221 hr assuming 1.5 x 10^6 hydroxyl radicals cm^{-3}, see Annex IIA point 2.10). Therefore it is considered that there is no risk of exposure to air and no further data are required.

5.2.4 Distribution modelling

No information available.

5.3 Aquatic Bioaccumulation

Table 22: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient n-octanol/water Japanese MAFF (12-Nousan-No. 8147, Part 2-9-11, 2000)	log P_{ow} = 3.52 at 25 ± 1 ºC pH: 7.2	Test substance: PGAI, Batch: R-4CM03G Purity: 99.3%	Lentz, N.R., 2005b (QNP-0002)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

No estimations are available.

5.3.1.2 Measured bioaccumulation data

Reference:	**Bioconcentration of [^{14}C]S-2188 by Bluegill Sunfish (*Lepomis macrochirus*)**
Author(s), year:	Panthani, A.M., Herczog, K.J.S., 2007
Report/Doc. number:	Report No. QNM-0018, Study No. 019492
Guideline(s):	OECD Guideline 305, US EPA FIFRA 165-4, US EPA OPPTS 850.1730
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	[pyrazolyl-5-^{14}C] S-2188: radiochemical purity: 98.6 - 99.6 %, batch: CFQ14368 Reference substances: unlabelled S-2188: purity 99.3 %, batch: R-4CM03G S-2188-OH: purity: 98.3 %, batch: CTS05014 S-2188-DC: purity: 99.9 %, batch: CTS04019 S-2188-CH_2OH-DC: purity: 100 %, batch: CTS05018 MCNI: purity: 99.9 %, batch: CTS05012 MPPZ: purity: 100 %, batch: CTS04003
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Number of organisms:	125 fish per test concentration and solvent control, 0.43 g fish/L/day (at initiation)
Weight, length:	2.1 (1.9 – 2.3) g, 56 (52 - 63) mm, n = 30
Type of test, duration:	Flow-through test, 28 d exposure period and 14 d depuration period
Applied concentrations:	
Nominal:	0 (solvent control), 0.005 and 0.05 mg a.s./L
Measured (mean):	- (solvent control), 0.00525 and 0.0479 mg a.s./L
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	

Water quality:	Well water, total hardness: 42 – 56 mg/L as $CaCO_3$
Temperature:	23 - 25 °C
pH:	6.8 – 7.6
O_2 content:	Exposure phase: 5.4 mg O_2/L (64 – 101% saturation) Depuration phase: 7.5 – 8.5 mg O_2/L (90 – 101% saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding:	Pelleted food: 1 % of biomass daily
Test parameters:	Samples were taken at day 0, 1, 3, 7, 14, 21 and 28 (exposure phase) and 1, 3, 7 and 14 (depuration phase). Concentration of [^{14}C] S-2188 equivalents in fish tissues were determined by LSC-method. Five fish for tissue analysis were removed from each test concentration and control at each sampling time. Additionally a lipid analysis (by chloroform/methanol extraction) was carried out on fish sampled at day 1, 3, 7, 14, 21 and 28 (exposure phase) and at day 1, 3, 7 and 14 (depuration phase). For chemical analysis (LSC, HPLC/RAM) of S-2188 in test solutions samples were taken at -2 and -1 d (pre-exposure phase), 0, 1, 3, 7, 14, 21 and 28 d (exposure phase) and 1, 3, 7 and 14 d (depuration phase). Daily observations were made of the appearance and behaviour of the fish. Other parameters like temperature, pH and dissolved oxygen concentrations were measured daily in each vessel.
Calculations/statistics:	BCF was calculated as ratio of [^{14}C] S-2188 equivalents concentration in water and [^{14}C] S-2188 equivalents concentration in fish tissues and as ratio of K_d (depuration constant) and K_u (uptake constant), rate constant K was determined by SigmaPlot™.
Findings:	
Analytical data – water:	The mean measured concentrations of [^{14}C] S-2188 equivalents were 94.3% (low concentration) and 89.5 % (high concentration) of nominal. HPLC/RAM analysis confirmed that S-2188 was stable in both test concentrations. In the high test concentration (42 µg/L), 82.4 – 95.5 % was determined as active substance. In the low test concentration (4.5 µg/L), 89.7 – 99.9 % was determined as active substance.
Lipid content:	No differences between male and female fish were noted. The steady state average lipid content of day 14, 21 and 28 was 1.96% (w/w, low concentration) and 1.94% (w/w, high concentration), respectively.
Analytical data – fish tissues (LSC):	See Table
BCF:	See Table

Table 23: Uptake, bioconcentration and depuration of [^{14}C] residues in the bluegill sunfish

Day	Mean concentration of [^{14}C] residues [ppm] (% of TRR)					
	Edible		Non-edible		Whole fish	
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L
Exposure phase						
1	0.311 (98.9)	3.047 (98.8)	0.685 (96.3)	6.408 (97.3)	0.501	4.824
3	0.541 (96.9)	4.607 (98.4)	1.574 (93.8)	13.28 (96.5)	1.101	9.071
7	0.708 (97.8)	5.698 (98.1)	2.214 (94.0)	18.04 (96.1)	1.470	12.18
14	0.692 (97.3)	8.057 (98.0)	2.197 (94.4)	22.27 (96.0)	1.505	15.84
21	0.683 (96.8)	6.749 (97.5)	2.064 (94.1)	19.26 (95.5)	1.437	13.40
28	0.755 (96.3)	6.641 (97.1)	2.271 (93.6)	18.95 (94.0)	1.562	13.28

Day	Mean concentration of [^{14}C] residues [ppm] (% of TRR)					
	Edible		Non-edible		Whole fish	
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L
Depuration phase						
1	0.524 (94.4)	4.0838 (96.2)	1.990 (90.8)	14.18 (93.9)	1.315	9.456
3	0.171 (87.9)	1.514 (91.8)	0.637 (88.1)	6.214 (90.4)	0.446	4.042
7	0.043 (71.5)	0.277 (74.6)	0.207 (75.1)	1.237 (77.1)	0.164	0.961
14	0.013 (51.1)	0.117 (61.0)	0.056 (57.4)	0.522 (67.5)	0.060	0.467

n.d…not detectable

Table 24: Uptake, bioconcentration and depuration of [^{14}C] S-2188 in the bluegill sunfish

Day	Mean concentration of [^{14}C] S-2188 [ppm] (% of TRR)							
	Edible		Non-edible		Whole body			
	0.005 mg/L	0.05 mg/L	0.005 mg/L	0.05 mg/L	0.005 mg/L		0.05 mg/L	
					ppm	BCF	ppm	BCF
Exposure phase								
1	0.042 (13.5)	0.29 (9.4)	0.041 (5.7)	0.366 (5.6)	0.042 (8.3)	9.1	0.328 (6.8)	0.0076
3	0.024 (4.3)	0.3 (6.4)	0.042 (2.5)	0.571 (4.2)	0.033 (3.0)	7.2	0.431 (4.8)	9.6
7	0.03 (4.1)	0.33 (5.7)	0.063 (2.7)	0.629 (3.3)	0.045 (3.1)	9.4	0.477 (3.9)	10.4
14	0.031 (4.4)	0.466 (5.7)	0.052 (2.2)	0.354 (1.5)	0.041 (2.8)	8.2	0.409 (2.6)	8.5
21	0.029 (4.1)	0.281 (4.1)	0.048 (2.2)	0.452 (2.2)	0.038 (2.7)	7.8	0.365 (2.7)	7.9
28	0.045 (5.8)	0.252 (3.7)	0.041 (1.0)	0.401 (2.0)	0.043 (2.8)	9.6	0.324 (2.4)	7.9
Depuration phase								
1	0.009 (1.6)	0.052 (1.2)	0.006 (0.3)	0.055 (0.4)	0.007 (0.6)	-	0.053 (0.6)	-
3	0.001 (0.7)	0.02 (1.2)	n.d.	0.031 (0.4)	0.001 (0.2)	-	0.025 (0.6)	-
7	0.001 (1.8)	0.006 (1.7)	0.001 (0.4)	0.011 (0.7)	0.001 (0.7)	-	0.008 (0.9)	-
14	0.001 (2.8)	0.006 (3.0)	0.001 (0.6)	0.008 (1.1)	0.001 (1.1)	-	0.007 (1.5)	-

n.d…not detectable

Table 25: Distribution of ^{14}C residues in whole fish samples

Concentration of ^{14}C residues [% TRR] in whole body										
	0.005 mg/L					0.05 mg/L				
Day	1	7	14	21	28	1	7	14	21	28
Exposure phase										
Extractable	97.1	95.1	95.1	94.8	94.3	97.8	96.6	96.5	96.0	94.8
Unextractable	2.9	4.9	4.9	5.2	5.7	2.2	3.4	3.5	4.0	5.2
S-2188	8.3	3.1	2.8	2.7	2.8	6.8	3.8	2.6	2.7	2.4
S-2188-OH	4.4	2.8	4.0	3.7	4.6	2.9	4.9	5.5	5.0	4.1
S-2188-DC	16.1	8.5	9.8	13.1	9.1	18.8	9.4	10.5	8.0	11.5
S-2188-DC conjugate	16.1	33.3	29.8	32.7	28.6	19.4	31.6	28.8	32.7	27.3
65 – 72 min [a]	27.8	14.8	15.9	9.1	12.4	22.3	15.4	14.2	16.0	18.6
Minor unknowns [b]	3.5	12.5	10.9	13.3	14.2	5.4	10.7	15.1	13.0	12.0
Others	20.8	19.0	19.6	16.8	19.8	22.0	19.5	15.9	16.6	17.0
Depuration phase										
Extractable	91.6	74.4	56.0	-	-	94.4	76.6	66.1	-	-
Unextractable	8.4	25.6	44.0	-	-	5.6	23.4	33.9	-	-
S-2188	0.6	0.7	1.1	-	-	0.6	0.9	1.5	-	-
S-2188-OH	0.9	n.d.	n.d.	-	-	2.8	n.d.	n.d.	-	-
S-2188-DC	7.4	n.d.	n.d.	-	-	7.6	2.1	n.d.	-	-

Concentration of ^{14}C residues [% TRR] in whole body										
	0.005 mg/L					0.05 mg/L				
Day	1	7	14	21	28	1	7	14	21	28
S-2188-DC conjugate	26.5	22.3	16.9	-	-	37.0	30.5	16.0	-	-
65 – 72 min [a]	13.5	7.9	10.4	-	-	16.2	9.8	12.3	-	-
Minor unknowns [b]	23.4	7.1	1.8	-	-	12.8	7.4	5.7	-	-
Others	16.7	12.6	2.2	-	-	14.3	11.1	0.6	-	-

n.d…not detectable
[a] A broad region of radioactivity containing multiple minor components. The retention times are characteristics of lipids and fatty acid esters.
[b] Minor unknowns consist of several components each less than 4% of the TRR, except for one component eluting at 19.4 minutes present in the depuration day 1 fish which consisted of 15.3% TRR (336 µg/kg) in the non-edible fraction and 11.9% TRR (156 µg/kg) in the whole fish.

Table 26: Summary of bioconcentration factors

	Bioconcentration factors (BCFs)					
	TRR			S-2188		
BCF	Edible	Non-edible	Whole body	Edible	Non-edible	Whole body
	0.005 mg/L (low concentration)					
Steady-state BCF	139	437	283	7	10	9
Uptake rate constant (Ku)	62.915	156.528	106.497	22.6	18.452	22.212
Depuration rate constant (Kd)	0.447	0.345	0.367	3.222	1.799	2.614
Kinetic BCF (BCFK) a	141	453	290	7	10	8
Lipid BCF b	102	168	144	5	4	4
	0.05 mg/L (high concentration)					
Steady-state BCF	149	431	289	7	9	8
Uptake rate constant (Ku)	62.663	149.356	104.849	13.658	17.92	15.638
Depuration rate constant (Kd)	0.439	0.347	0.369	1.86	1.687	1.755
Kinetic BCF (BCFK) a	143	430	284	7	11	9
Lipid BCF b	113	166	149	6	3	4

[a] Ratio Ku/Kd
[b] Steady-state BCF/average of steady-state lipid content [% tissue wet weight]

Conclusion: S-2188 was stable under the test conditions and reached the steady-state plateau at day 28 of exposure.
The active substance S-2188 accumulated in whole fish with steady-state BCF values in whole fish tissues of 8 and 9. In non-edible portions the BCF of 9 and 10 were determined. BCF values for the total ^{14}C residues (TRR) were determined to be 283 and 289 for whole fish, and 437 and 431 for non-edible portions.
The modelled uptake rate constants (K_u) for S-2188 in whole fish tissues ranged from 15.6 to 22.2 per day, depuration constants (K_d) for S-2188 in whole fish tissues ranged from 1.76 to 2.61 per day. Greater than 95% of the ^{14}C residues were eliminated during the depuration phase (within 14 d). The depuration half-life (CT_{50}) was < 1 day.
S-2188 was extensively metabolized in fish. The major residues were a glucuronic acid conjugate of the metabolite S-2188-DC and the metabolite S-2188-DC itself. The concentrations of S-2188-DC conjugate and S-2188-DC were determined to be between 16.1 to 33.3 % TRR and 8.0 to 18.8 % TRR in whole fish during the exposure phase, respectively.

5.3.2 Summary and discussion of aquatic bioaccumulation

Fenpyrazamine has a log P_{OW} of 3.52 and therefore a fish bioconcentration study is triggered. Based on the fish bioaccumulation study (Panthani, A.M., Herczog, K.J.S., 2007) with *L. macrochirus* a BCF (whole fish) of 9 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain.
In DAR BCF was determined only for viscera and fillet, but was not corrected by lipid content.

The active substance was extensively metabolized in fish and the residues were eliminated quickly (CT_{50} < 1 d).

The major residues were a glucuronic acid conjugate of the metabolite S-2188-DC and the metabolite S-2188-DC itself. The concentrations of S-2188-DC conjugate and S-2188-DC were determined to be between 16.1 to 33.3 % TRR and 8.0 to 18.8 % TRR in whole fish during the exposure phase, respectively.

The bioaccumulation potential of all major metabolites in water and sediment (S-2188-OH, S-2188-DC and MCNI) is also assumed to be low, due to log P_{OW} values clearly lower than 3. Thus, it can be concluded that the risk of bioaccumulation of the major metabolites in the aquatic ecosystem is acceptable.

5.4 Aquatic toxicity

Standard toxicity studies on fish, aquatic invertebrates and algae with Fenpyrazamine were performed. Fenpyrazamine is toxic (LC_{50}/EC_{50} is ≥ 1 mg and < 10 mg/L) to the used standard fresh water test species. The most sensitive species is the algae *Pseudokirchneriella subcapitata* with an E_bC_{50} of 0.42 mg a.s./L.

Additional toxicity studies on marine and freshwater species (fish, aquatic invertebrates, bivalve, algae and aquatic macrophytes) were submitted by the applicant for the re-assessment of the classification of Fenpyrazamine. These studies were not submitted by the applicant for the EU assessment of the active substance Fenpyrazamine. Hence, the assessment and validation of the studies are not EU peer-reviewed.

Table 27: Summary of relevant information on aquatic toxicity

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OECD 203, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Oncorhynchus mykiss* Rainbow trout	flow-through	96 hr	mm	Mortality	1.1	5.2	Cafarella, M.A., 2006a Report No.: QNW-0002 Study No. 13048.6504
OECD 203, OPPTS 850.1075, EU Directive 92/69/EEC C.1	*Lepomis macrochirus* Bluegill sunfish	flow-through	96 hr	mm	Mortality	3.4	5.4	Cafarella, M.A., 2006b Report No.: QNW-0006 Study No. 13048.6505
OPPTS 850.1075	*Cyprinodon variegatus* Sheepshead minnow	static	96 hr	mm	Mortality	1.9	> 3.9	Fournier, A.E., 2010a [a] Report No.: QNW-0048 Study No. 12079.6301
OECD 210, OPPTS 850.1400	*Oncorhynchus mykiss* Rainbow trout	flow-through	90 d	mm	Fry survival Growth	0.37	> 0.75	Cafarella, M.A., 2006c Report No.: QNW-0011 Study No. 13048.6506
OPPTS 850.1400	*Cyprinodon variegatus* Sheepshead minnow	flow-through	33 d	mm	Fry survival Growth	1.2 0.062	> 1.2	Lee, M. R., 2010° [a] Report No.: QNW-0050 Study No. 12079.6292
OECD 202, OPPTS 850.1010, JMAFF No.12-Nousan-8147, Daphnia Acute Immobilisation Test (2-7-2-1), EU Directive 92/69/EEC C.2	*Daphnia magna* Water flea	flow-through	48 hr	mm	Immobility	< 0.61	5.5	Putt, A.E., 2006a Report No.: QNW-0007 Study No. 13048.6507
OPPTS 850.1035	*Americamysis bahia* Mysid	static	96 hr	mm	Immobility	0.29	0.83	Fournier, A.E., 2010b [a] Report No.: QNW-0047 Study No. 12079.6303
OECD 211, FIFRA 72-4, OPPTS 850.1300	*Daphnia magna* Water flea	flow-through	21 d	mm	Mortality adults Reproduction Growth	1.4 0.34 0.34	> 1.4 1.1 n.d.	Putt, A.E., 2006b Report No.: QNW-0012 Study No. 13048.6508
OPPTS 850.1350	*Americamysis bahia* Mysid	flow-through	28 d	mm	Mortality Reproduction Growth	0.098 0.098 0.024	> 0.098	Lee, M.R., 2010b [a] Report No.: QNW-0049 Study No. 12709.6293

Method	Test organism	Test condition	Exp. time	Test conc.	Results			Reference
					Endpoint	NOEC [mg a.s./L]	EC_{50}/LC_{50} [mg a.s./L]	
OPPTS 850.1735, OECD 218, EPA Test Method 100.4	*Hyalella azteca* Freshwater amphipod	static-renewal	42 d	mm	Mortality Reproduction Growth	10 mg a.s./kg	61 mg a.s./kg 55 mg a.s./kg > 94 mg a.s./kg	Picard, C. R., 2010a [a] Report No.: QNW-0052 Study No. 12079.6290
OPPTS 850.1740, Guideline Series 850.0000	*Leptocheirus plumulosus* Estuarine amphipod	static-renewal	28 d	mm	Mortality Reproduction Growth	12 mg a.s./kg 6.6 mg a.s./kg 6.6 mg a.s./kg	17 mg a.s./kg 9.4 mg a.s./kg > 12 mg a.s./kg	Picard, C. R., 2010b [a] Report No.: QNW-0053 Study No. 12079.6291
OPPTS 850-1025	*Crassostrea virginica* Oyster	flow-through	96 hr	mm	Shell deposition	0.24	0.66	York, D. O., 2010 [a] Report No.: QNW-0046 Study No. 12079.6302
OECD 201, JMAFF No.12-Nousan-8147, Alga Growth Inhibition Test (2-7-7), OPPTS 850.5400, EU Directive 92/69/EEC C.3	*Pseudokirchneriella subcapitata* Freshwater green alga	static	96 hr	mm	Biomass Growth rate Cell density	0.22 0.22 0.053	0.42 > 0.9 0.19	Hoberg, J.R., 2006a Report No.: QNW-0004 Study No. 13048.6509
OPPTS 850.5400	*Navicula pelliculosa* Freshwater diatom	static	96 hr	mm	Yield [b] Growth rate [b] Cell density	0.0049 0.074 0.0049	0.008 0.202 0.011	Softcheck, K. A., 2010b [a] Report No.: QNW-0057 Study No. 12079.6304
OPPTS 850.5400	*Skeletonema costatum* Marine diatom	static	96 hr	mm	Yield [b] Growth rate [b] Cell density	0.011	0.022 0.034 0.027	Softcheck, K. A., 2010a [a] Report No.: QNW-0056 Study No. 12079.6307
OPPTS 850.4400	*Lemna gibba* Duckweed	static-renewal	7 d	mm	Frond number Biomass Growth rate	0.15 0.06 0.36	1.5 1.2 4.9	Softcheck, K. A., 2010d [a] Report No.: QNW-0055 Study No. 12079.6305

[a] Study was newly submitted and was not included in the previous proposal.
[b] Endpoint was calculated by the RMS using the software ToxRat®

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference:	**S-2188 Technical Grade – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions**
Author(s), year:	Cafarella, M. A., 2006a
Report/Doc. number:	Report No. QNW-0002, Study No. 13048.6504
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Rainbow trout (*Oncorhynchus mykiss*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length:	1.7 g (range 0.98 – 2.4 g) and 5.7 cm (range 4.7 – 6.8 cm), n = 30
Loading:	0.18 g fish/L solution
Type of test, duration:	Flow-through test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.5, 1.0, 2.0, 4.0 and 8.0 mg a.s./L
Measured (mean):	- (control and solvent control), 0.51,1.1, 2.1, 3.8 and 7.8 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Well water, total hardness: 50 mg/L as $CaCO_3$
Temperature:	12 – 13 °C
pH:	7.0 (0 h, new solution), 7.2 (96 h, aged solution)
O_2 content:	8.5 – 10.8 mg O_2/L (80 – 103% saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 3, 6, 24, 48, 72 and 96 hours. For chemical analysis (LC/MS/MS) of S-2188 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 94 to 106% of nominal.
Behavioural effects:	Controls and concentration levels up to 1.1 mg a.s./L: No sublethal effects were reported over the whole test period. At test concentration 2.1 mg a.s./L following symptoms were noted after 48 hours: Lethargic behaviour remained at water surface. At test concentration 3.8 mg a.s./L following symptoms were noted after 3 hours: Lethargic behaviour, loss

of equilibrium, remained at water surface. At test concentration 7.8 mg a.s./L following symptoms were noted after 3 hours: Complete loss of equilibrium.
Thus the NOEC was 1.1 mg a.s./L based on sublethal effects.

Mortality: See Table

Table 28: Effects on rainbow trout (O. mykiss) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	Cumulative mean mortality [%]					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.51	0	0	0	0	0	0
1.1	0	0	0	0	5	5
2.1	0	0	0	0 [ae]	0	0 [ae]
3.8	0 [ace]	0 [abc]	0 [abcd]	0 [abce]	0 [abc]	5 [abc]
7.8	0 [c]	100	100	100	100	100
NOEC = 1.1 mg a.s./L						
LC_{50} (96 h) = 5.2 mg a.s./L (95 % C.I. 3.8 – 7.8 mg a.s./L)						

[a] lethargic behaviour
[b] partial loss of equilibrium
[c] complete loss of equilibrium
[d] dark in colour
[e] fish on surface of test solution

Conclusion: 96 h LC_{50} = 5.2 mg a.s./L
96 h NOEC = 1.1 mg a.s./L
based on mean measured concentrations

Comment RMS: The observed mortality of 5% at the test concentration of 1.1 mg a.s./L was not considered for the NOEC determination because the effect did not establish a dose-response relationship. Hence, the NOEC was determined to be 1.1 mg a.s./L, based on behavioural effects (lethargy and loss of equilibrium) on fish at application rates of 2.1 mg a.s./L and higher.

Reference:	**S-2188 Technical Grade – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-through Conditions**
Author(s), year:	Cafarella, M. A., 2006b
Report/Doc. number:	Report No. QNW-0006, Study No. 13048.6505
Guideline(s):	OECD Guideline 203, US EPA OPPTS 850.1075, EC Guideline Annex V - Method C.1
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Bluegill Sunfish (*Lepomis macrochirus*)
Number of organisms:	10 fish per replicate, 2 replicates per treatment, control and solvent control
Weight, length:	1.7 g (range 1.1 – 2.9 g) and 4.7 mm (range 4.0 – 5.6 mm), n = 30
Loading:	0.17 g fish/L solution
Type of test, duration:	Flow-through test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 1.0, 2.0, 4.0, 8.0 and 16.0 mg a.s./L
Measured (mean):	- (control and solvent control), 0.88, 1.8, 3.4, 6.9 and 12.0 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Well water, total hardness: 48 mg/L as $CaCO_3$
Temperature:	22 ± 1 °C
pH:	7.2 ± 0.1 (0 h, new solution), 7.3 ± 0.1 (96 h, aged solution)
O_2 content:	7.6 – 9.5 mg O_2/L (94 – 108% saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Mortality and sublethal effects were assessed after 0, 3, 6, 24, 48, 72 and 96 hours. For chemical analysis (LC/MS/MS) of S-2188 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily in both vessels of each treatment.
Statistics:	LC_{50}: Binominal probability, NOEC: Directly from raw data
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 77 to 91% of nominal concentrations.
Behavioural effects:	Controls and concentration levels up to 3.4 mg a.s./L: No sublethal effects were reported over the whole test period. At test concentration 6.9 mg a.s./L following symptoms were noted after 3 hours: Partial and complete loss of equilibrium. At test concentration 12.0 mg a.s./L following symptoms were noted after 3 hours: Complete loss of equilibrium. Thus the NOEC was 3.4 mg a.s./L based on sublethal effects.
Mortality:	See Table

Table 29: Effects on bluegill sunfish (*L. macrochirus*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	Cumulative mortality [%]					
	3 hours	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0
0.88	0	0	0	0	0	0
1.8	0	0	0	0	0	0
3.4	0	0	0	0	0	0
6.9	0 [ab]	0 [bc]	25 [c]	30 [c]	70 [c]	85 [c]
12.0	0 [c]	100	100	100	100	100
NOEC = 3.4 mg a.s./L						
LC_{50} (96 h) = 5.4 mg a.s./L (95 % C.I. 3.4 – 6.9 mg a.s./L)						

[a] lethargic behaviour
[b] partial loss of equilibrium
[c] complete loss of equilibrium

Conclusion: 96 h LC_{50} = 5.4 mg a.s./L
96 h NOEC = 3.4 mg a.s./L
based on mean measured concentration

Reference:	**V-10135 T.G. (S-2188 T.G.) Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Conditions**
Author(s), year:	Fournier, A. E., 2010a
Report/Doc. number:	Report No. QNW-0048, Study No. 12079.6301
Guideline(s):	US EPA OPPTS 850.1075
GLP:	Yes
Deviations:	The protocol states that the water temperature during exposure will be maintained at 22 ± 1 ºC. During this study, daily temperature measurements ranged from 22 ºC to 24 ºC. The minimum/maximum thermometer recorded a maximum temperature of 24 ºC. Since control performance met all protocol requirements, this deviation had no impact on the results or interpretation of this test.
Validity:	Acceptable

Material and methods:
Test substance: V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species: Sheepshead minnow (*Cyprinodon variegatus*)
Number of organisms: 10 fish per replicate, 2 replicates per treatment, control
Weight, length: 0.31 g (range 0.17 – 0.51 g) and 27 mm (range 23 – 33 mm), n = 30
Loading: 0.21 g fish/L solution
Type of test, duration: Static test, 96 hours
Applied concentrations:
Nominal: 0 (control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L
Measured (mean): - (control), 0.24, 0.50, 1.0, 1.9 and 3.9 mg a.s./L
Solvent: None
Test conditions:
Water quality: Natural seawater (filtered), salinity: 20 - 21‰

Temperature:	22 - 24 °C
pH:	7.4 – 7.8
O_2 content:	5.1 – 7.6 mg O_2/L (> 60% saturation)
Light regime:	16 hours light / 8 hours darkness, light intensity: 830 – 1000 lux
Test parameters:	Mortality and sublethal effects were assessed after 0, 6, 24, 48, 72 and 96 hours. For chemical analysis of V-10135 in test solutions samples were taken at test initiation (0 h) and test termination (96 h) from all treatment groups and the control. Measurement of pH, temperature, salinity and dissolved oxygen concentrations were made at initiation and once daily in each test vessel.
Statistics:	LC_{50}, NOEC: Directly from raw data, empirically estimated.
Findings:	
Analytical data:	Over the whole test period the mean measured concentrations were in the range from 76 - 80% of nominal.
Behavioural effects:	Controls and concentration levels up to 1.9 mg a.s./L: No sublethal effects were reported over the whole test period. At test concentration 3.9 mg a.s./L following symptoms were noted: Lethargic behaviour, fish remained at bottom of the test vessel, partial or complete loss of equilibrium. Thus the NOEC was 1.9 mg a.s./L based on sublethal effects.
Mortality:	See Table

Table 30: Effects on sheepshead minnow (*C. variegatus*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	Cumulative mean mortality [%]				
	6 hours	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0	0
0.24	0	0	0	0	0
0.50	0	0	0	0	0
1.0	0	0	0	0	0
1.9	0	0	0	0	0
3.9	0 [a]	0[abd]	10[abd]	15 [ac]	20 [ac]
NOEC = 1.9 mg a.s./L					
LC_{50} (96 h) > 3.9 mg a.s./L (95 % C.I. n.a.)					

[a] partial loss of equilibrium
[b] complete loss of equilibrium
[c] lethargic behaviour
[d] fish on bottom of the test vessel

Conclusion:	96 h LC_{50} > 3.9 mg a.s./L 96 h NOEC = 1.9 mg a.s./L based on mean measured concentrations

5.4.1.2 Long-term toxicity to fish

Chronic toxicity to fish (IIA 8.2.2)

Prolonged toxicity (21 day exposure) to fish (IIA 8.2.2.1)

No study submitted. The requirement for data on the chronic effects of fenpyrazamine on juvenile fish has been addressed by the submission of an early life stage toxicity test (ELS-test) with the freshwater species rainbow trout (*O. mykiss*) and the marine species sheepshead minnow (*C. variegatus*).

Fish early life stage toxicity test (IIA 8.2.2.2)

Reference:	**S-2188 Technical Grade – Early Life Stage Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*)**
Author(s), year:	Cafarella, M. A., 2006c
Report/Doc. number:	Report No. QNW-0011, Study No. 13048.6506
Guideline(s):	OECD Guideline 210, US EPA OPPTS 850.1400
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Rainbow Trout (*Oncorhynchus mykiss*)
Number of organisms:	2 replicates per test concentration, control and solvent control. 50 eggs per egg incubation cup, after completion of hatch larvae were thinned to 20 individuals per aquarium. At the highest nominal treatment level, an additional of 10 eggs were selected from each incubation cup and suspended in each exposure aquarium. For the recovery/reversibility test group 20 (high dose recovery group – HDR) larvae per aquarium from the highest test concentration were transferred to clean dilution water containing no S-2188.
Age:	Freshly fertilized eggs, 2.5 hours old
Type of test, duration:	Flow-through test, 90 days (60 days post hatch)
Applied concentrations:	
Nominal:	0 (control and solvent control), 50, 100, 200, 400 and 800 µg/L
Measured (mean):	- (control and solvent control), 50, 100, 190, 370 and 750 µg/L
Solvent	Dimethylformamide (DMF, CAS No. 68-12-2), 0.01 mL/L
Test conditions:	
Water quality:	Well water, total hardness: 32 – 58 mg/L as $CaCO_3$
Temperature:	11 – 13 °C
pH:	6.9 – 7.8 during the total test period
O_2 content:	8.2 – 11.1 mg O_2/L (76 – 103 % saturation)
Light regime:	Continuous darkness during incubation phase and prior to the larval development to the swim-up stage. From swim-up phase onwards: 16 hours light / 8 hours darkness, sudden transitions were avoided
Feeding	Larvae were fed of live brine shrimp nauplii (*Artemia salina*) 3 times daily beginning on day 11 post-hatch. Larvae were not fed during the final 48 hours of the test.

	Residual food and fecal matter were brushed and siphoned when necessary in order to minimise microbiological growth.
Test parameters:	Abnormal appearance and behaviour of larvae were assessed daily. Number of surviving larvae was estimated at least twice a week. At test termination the length and the weight were determined. Determined endpoints were: Hatching success, overall fry survival (fry survival before and after thinning), mean length, wet and dry weight. Temperature, pH and dissolved oxygen concentration were measured daily. Total hardness, alkalinity and specific conductance were measured weekly. Analytical measurements (LC/MS/MS) of S-2188 in test solutions samples were taken at 0, 7, 14, 21, 28, 35, 42, 49, 56, 63, 73, 77, 84 and 90 days.
Statistics:	If control and solvent control can be pooled: t-Test Testing for normal distribution: Shapiro Wilks' Test Homogeneity of variance, data for embryo viability: Bartlett's Test Larval survival: Bonferroni's t-Test, Kruskal-Wallis Test and ANOVA Time to hatch, hatching success, mean length, mean dry and wet weight: Williams test with previous acrisine transformation
Findings:	
Analytical data:	Overall mean measured concentrations in test media were 93 – 100 % of nominal.
Biological observation:	Total length of larvae: No significant differences were observed in the mean total length of the larvae from the treatment groups (57.3 – 57.6 mm) and the pooled control groups (57.6 mm). Time to hatch: In control and all treatment levels hatching began on day 4 and continued until day 5. Morphological and behavioural effects: Over the total test period no morphological and behavioural effects were observed. High dose recovery group: During the first few weeks of clean water exposure sublethal effects like lethargy, darkened pigmentations and haemorrhage were observed in several larval fish. These were consistent with observation made at the highest treatment level tested. As the exposure in clean water continued these sublethal effects diminished and were no longer evident by test day 57. Based on the observations it can be assumed that larval fish exposed to concentrations of S-2188 at ≤ 0.75 mg a.s./L during embryonic development will recover.
Effects:	See Table

Table 31: Hatching success and fry survival

Fenpyrazamine [mg a.s./L] (mean measured)	Mean embryo viability [%] [a] phd -11	Mean hatching survival [%] [b] phd 0	Mean larvae survival [%] [c] phd 60
Control	62	99	98
Solvent control	67	100	98
Pooled control [d]	64	100	98
0.05	70	100	98
0.1	69	99	100
0.19	70	99	98
0.37	61	98	100
0.75	68	96 *	45 *
HDR	n.a.	n.a.	95
NOEC = 0.37 mg a.s./L			
LOEC = 0.75 mg a.s./L			
MATC = 0.53 mg a.s./L			

phd…post hatch day, HDR…high dose recovery group (0.75 mg a.s./L)
[a] Mean viability of embryos assessed on test day 19 (phd -11).
[b] Mean survival at complete hatch was observed on test day 30 (phd 0).
[c] Survival of larvae on test day 90 (phd 60).
[d] No statistically significant difference between dilution control and solvent control (t-Test)
* Significantly different compared to the pooled control (alpha = 0.05)

Table 32: Length and Weight

fenpyrazamine [mg a.s/L] (mean measured)	Mean length [mm] (SD) phd 60	Mean dry weight [mg] (SD) phd 60
Control	57.7 (2.74)	0.337 (0.0637)
Solvent control	57.5 (4.76)	0.338 (0.102)
Pooled control [a]	57.6 (3.86)	0.337 (0.0842)
0.05	57.3 (4.24)	0.332 (0.0831)
0.1	57.6 (3.14)	0.335 (0.0714)
0.19	57.3 (2.93)	0.333 (0.0674)
0.37	57.3 (2.64)	0.335 (0.0568)
0.75	55.8 (3.33) [b]	0.336 (0.068) [b]
HDR	57.6 (3.35)	0.345 (0.0868)
NOEC = 0.37 mg a.s./L		
LOEC = 0.75 mg a.s./L		
EC_{50} > 0.75 mg a.s./L		

phd…post hatch day, HDR…high dose recovery group (0.75 mg a.s./L)
[a] No statistically significant difference between dilution control and solvent control.
[b] Excluded from further statistical analyses due to a significant effect on larval survival.

Conclusion: 90 d NOEC = 0.37 mg a.s./L (fry survival)
90 d LOEC = 0.75 mg a.s./L
90 d EC_{50} > 0.75 mg a.s./L
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.) Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*) Following OPPTS Draft Guideline 850.1400**
Author(s), year:	Lee, M. R, 2010a
Report/Doc. number:	Report No. QNW-0050, Study No. 12709.6292
Guideline(s):	US EPA OPPTS 850.1400
GLP:	Yes
Deviations:	According to the study protocol, sheepshead embryos that are less than 30 hours old will be used to initiate the early life stage test. For this study, at test initiation the embryos were 30 hours old. Embryos were purchased from a commercial supplier and were added to the test system as early as possible, following temperature acclimation. This deviation did not have a negative effect on the results or interpretation of this study. According to the study protocol, when hatch is designated as being complete, 10 fry will be impartially selected and transferred to the respective test aquaria. For this study, following hatch 11 fry were inadvertently transferred to the D replicate of the 0.047 mg/L nominal test level. The organisms in this vessel did not exceed the recommended loading rate nor was their growth affected. This deviation did not have a negative impact on the results or interpretation of this study.
Validity:	Acceptable

Material and methods:	
Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Sheepshead minnow (*Cyprinodon variegatus*)
Number of organisms:	4 replicates per test concentration and control. 30 eggs per egg incubation cup, after completion of hatch (day 5) larvae were thinned to 10 individuals per replicate, 40 organisms per treatment level or control.
Age:	Freshly fertilized eggs, 30 hours old (at test initiation)
Type of test, duration:	Flow-through test, 33 days (28 days post hatch)
Applied concentrations:	
Nominal:	0 (control), 0.047, 0.094, 0.19, 0.38, 0.75 and 1.5 mg a.s./L
Measured (mean):	- (control), 0.039, 0.062, 0.15, 0.32, 0.58 and 1.2 mg a.s./L
Solvent	None
Test conditions:	
Water quality:	Natural seawater (filtered), salinity: 20 – 21 ‰
Temperature:	24 - 26 °C
pH:	7.4 – 7.9 during the total test period
O_2 content:	5.7 – 8.9 mg O_2/L (> 60 % saturation)
Salinity:	20 – 21 ‰
Light regime:	16 hours light / 8 hours darkness, light intensity: 540 – 710 lux
Feeding	Beginning on day 5 (day 0 post-hatch) larvae were fed of live brine shrimp nauplii (*Artemia salina*) 3 times daily. Larvae were not fed during the final 24 hours of the test.
Test parameters:	Dead and live embryos were counted daily until the day of hatch. Completion of hatch was considered to be exposure day 5, when no unhatched viable embryos remained in any control embryo incubation

cup. Calculation of percent embryo hatching success was based on the number of live, dead and deformed larvae per incubation cup after hatching was completed (day 5) compared to the number of embryos per cup on test day 0.
The 28-day post-hatch larval exposure was initiated on day of hatch (test day 5). The behaviour and appearance of the larval fish were observed daily. Larval survival was estimated daily throughout the post-hatch period. At test termination the length and the weight were determined.
Determined endpoints were: Hatching success, overall fry survival (fry survival before and after thinning), mean length, wet and dry weight.
Temperature, pH, salinity and dissolved oxygen concentration were measured at test initiation and daily thereafter.
Analytical measurements of S-2188 in test solutions samples were taken at 0, 5, 12, 13, 19, 26 and 33 days.

Statistics: Testing for normal distribution: Shapiro Wilks' Test
Testing of homogeneity of variance: Bartlett's Test
Comparison of performance at each treatment level with the control organisms: Kruskal-Wallis Test (data for larval survival and hatch) and Dunnetts' Test (mean length, mean dry and wet weight)

Findings:

Analytical data: Overall mean measured concentrations in test media were 66 - 84 % of nominal.

Effects: See Table

Table 33: Hatching success and fry survival

Fenpyrazamine [mg a.s./L] (mean measured)	**Mean embryo hatching success [%] [a]**	**Normal fry at hatch [%] [b] phd 0**	**Mean larvae survival [%] [c] phd 28**
Control	95	100	100
0.039	92	100	100
0.062	92	100	100
0.15	92	100	100
0.32	93	100	100
0.58	93	99	100
1.2	98	100	95
NOEC = 1.2 mg a.s./L			
LOEC > 1.2 mg a.s./L			

phd…post hatch day
[a] Mean hatching success of embryos assessed on test day 5 (competition of hatch).
[b] Mean survival at complete hatch was observed on test day 5 (phd 0).
[c] Survival of larvae on post hatch day 28.

Table 34: Length and Weight

Fenpyrazamine [mg a.s/L] (mean measured)	Mean length [mm] (SD) phd 28	Mean dry weight [mg] (SD) phd 28
Control	24.0 (0.29)	0.053 (0.0035)
0.039	23.3 (0.49)	0.048 (0.0043)
0.062	23.5 (0.36)	0.0506 (0.0031)
0.15	22.9 (0.16) *	0.0472 (0.0014)
0.32	22.7 (0.72) *	0.0498 (0.0058)
0.58	21.6 (0.67) *	0.0417 (0.005) *
1.2	20.3 (0.52) *	0.0369 (0.0037) *
NOEC	0.062 mg a.s./L	0.32 mg a.s./L
LOEC	0.15 mg a.s./L	0.58 mg a.s./L
EC_{50}	> 1.2 mg a.s./L	> 1.2 mg a.s./L

phd…post hatch day, SD…Standard deviation
* Statistically significant compared to the control data based on Dunnetts'Test

Conclusion: 33 d NOEC = 0.062 mg a.s./L (growth)
33 d LOEC = 0.15 mg a.s./L (growth)
33 d EC_{50} > 1.2 mg a.s./L
based on mean measured concentrations

Fish life cycle test (IIA 8.2.2.3)

No study was submitted. The BCF of fenpyrazamine is 9 and thus clearly less than 1000. In addition there was more than 95 % elimination of fenpyrazamine residues in fish in 14 days during the depuration phase of the fish bioaccumulation study.

The acute toxicity to fish was determined to be greater than 0.1 mg/L (LC_{50} = 5.2 mg a.s./L) and the persistence in water and sediment was observed to be below the trigger of DT_{90} > 100 d.

On this basis, a fish life-cycle test is not required and therefore was not conducted.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Reference:	**S-2188 Technical Grade – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Flow-Through Conditions**
Author(s), year:	Putt, A. E., 2006a
Report/Doc. number:	Report No. QNW-0007, Study No. 13048.6507
Guideline(s):	OECD Guideline 202, US EPA OPPTS 850.1010, JMAFF No.12-Nousan-8147, Daphnia Acute Immobilisation Test (2-7-2-1), EC Guideline Annex V - Method C.2
GLP:	Yes
Deviations:	- The temperature measured on test day 2 was between 19 and 22 °C instead of 20 ± 1 °C as stated in the protocol. The performance of the control organisms was satisfactory according to the given criteria. Therefore, the deviation was considered not to have an adverse effect on the results of the study.
Validity:	Acceptable

Material and methods:

Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	2 replicates each with 10 daphnids per treatment, control and solvent control
Age:	First instar, ≤ 24 hours old
Type of test, duration:	Flow-through test, 48 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.75, 1.5, 3.0, 6.0 and 12 mg a.s./L
Measured (mean):	- (control and solvent control), 0.61, 1.2, 2.2, 3.8 and 8.0 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL DMF/L
Test conditions:	
Water quality:	Fortified well water, total hardness: 160 - 170 mg/L as $CaCO_3$
Temperature:	19 - 22 °C
pH:	8.3 (0 - 48 h)
O_2 content:	8.2 – 9.1 mg O_2/L (90 – 100 % saturation)
Light regime:	16 hours light / 8 hours darkness
Test parameters:	Immobility and sublethal effects were assessed after 0, 24 and 48 hours. For chemical analysis (LC/MS/MS) of S-2188 in the test media samples were taken at test initiation (0 h) and termination (48 h). The water samples were analysed by automated injection into the LC/MS/MS instrument without centrifugation. Measurements of pH, temperature and dissolved oxygen concentrations were made at initiation and once daily. Total hardness, total alkalinity and specific conductance were measured at test initiation.
Statistics:	EC_{50}: Probit analysis, NOEC: Directly from the raw data
Findings:	
Analytical data:	The overall mean measured concentration ranged from 63 – 82 % of nominal concentrations.

Effects: After 48 hours no immobilisation was observed in the control, solvent control and in test concentrations up to 2.2 mg/L. At 3.8 and 8.0 mg/L the immobilisation was between 5 and 95 %. Sublethal effects (lethargy) were observed at all treatment groups.
Thus the NOEC was determined to be < 0.61 mg/L and the EC_{50} was 5.5 mg/L.

Table 35: Effects on daphnids (*D. magna*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	Mean cumulative immobilized organisms [%]	
	24 hours	48 hours
Control	0	0
Solvent control	0	0
0.61	0	0
1.2	0	0
2.2	0	0
3.8	0	5
8.0	45	95
NOEC < 0.61 mg a.s./L		
EC_{50} (48 h) = 5.5 mg a.s./L (95 % C.I. 4.7 – 6.5 mg a.s./L)		

Conclusion: 48 h EC_{50} = 5.5 mg a.s./L
48 h NOEC < 0.61 mg a.s./L
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.) Acute Toxicity to Mysid (*Americamysis bahia*), Under Static Conditions, Following OPPTS Guideline 850.1035**
Author(s), year:	Fournier, A. E., 2010b
Report/Doc. number:	Report No. QNW-0047, Study No. 12709.6303
Guideline(s):	US EPA OPPTS 850.1035
GLP:	Yes
Deviations:	None relevant
Validity:	Acceptable

Material and methods:
Test substance: V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species: Mysids (*Americamysis bahia*, formerly *Mysidopsis bahia*)
Number of organisms: 2 replicates each with 10 mysids per treatment and control.
Age: First instar, < 24 hours old
Type of test, duration: Static test, 96 hours
Applied concentrations:
Nominal: 0 (control), 0.31, 0.63, 1.3, 2.5 and 5.0 mg a.s./L
Measured (mean): - (control), 0.29, 0.62, 1.2, 2.3 and 4.6 mg a.s./L
Solvent: None
Test conditions:
Water quality: Natural seawater (filtered), salinity: 20 – 21 ‰
Temperature: 24 - 25 °C
pH: 7.4 – 7.9 (0 - 96 h)

O_2 content:	5.1 – 7.7 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness, light intensity: 710 – 990 lux
Feeding:	Live brine shrimp nauplii (*Artemia salina*) were added to each test vessel containing live test organisms once daily during the exposure period.
Test parameters:	Immobility and sublethal effects were assessed at test initiation and at each subsequent 24-hour interval until test termination (96 h). Measurements of pH, temperature, salinity and dissolved oxygen concentrations were made at initiation and once daily. For chemical analysis (HPLC/UV) of S-2188 in the test media samples were taken at test initiation (0 h) and termination (96 h).
Statistics:	EC_{50}: Binominal probability, NOEC: Directly from the raw data, empirically estimated
Findings:	
Analytical data:	The overall mean measured concentration ranged from 92 - 98 % of nominal concentrations.
Effects:	See Table

Table 36: Effects on mysids (*A. bahia*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	**Mean cumulative immobilised organisms [%]**			
	24 hours	**48 hours**	**72 hours**	**96 hours**
Control	0	0	0	0
0.29	0	0	0	0
0.62	0	5	5	5
1.2	85	100	100	100
2.3	100	100	100	100
4.6	100	100	100	100
NOEC = 0.29 mg a.s./L				
EC_{50} (96 h) = 0.83 mg a.s./L (95 % C.I. 0.62 – 1.2 mg a.s./L)				

Conclusion:	96 h EC_{50} = 0.83 mg a.s./L 96 h NOEC = 0.29 mg a.s./L based on mean measured concentrations

5.4.2.2 Long-term toxicity to aquatic invertebrates

Reference:	**S-2188 Technical Grade – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions**
Author(s), year:	Putt, A.E., 2006b
Report/Doc. number:	Report No. QNW-0012, Study No. 13048.6508
Guideline(s):	OECD Guideline 211, FIFRA 72-4, OPPTS 850.1300
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:	
Test substance:	Fenpyrazamine (S-2188) technical grade, purity: 94.7 %, batch: 030-050914-1G
Test species:	Water flea (*Daphnia magna*)
Number of organisms:	4 replicates per treatment group and controls, each with 10 daphnids
Age:	First instar, < 24 hours old
Type of test, duration:	Flow-through test, 21 d
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.1, 0.2, 0.4, 0.8 and 1.6 mg a.s./L
Measured (mean):	- (control and solvent control), 0.085, 0.18, 0.34, 0.76 and 1.4 mg a.s./L
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	
Water quality:	Fortified well water, hardness: 170 – 180 mg/L as $CaCO_3$
Temperature	19 – 21 °C
pH	7.9 – 8.3
O_2 content:	5.6 – 8.7 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness
Feeding	Daphnids were fed with *Ankistrodesmus falcatus* suspension (4 x 10^7 cells/mL), and 1 mL of a yeast, cereal leaves and digested flaked fish food suspension, three times daily.
Test parameters:	Parent mobility, mortality and abnormal behaviour were observed daily. Reproduction (mean time to first brood, age at first brood, offspring per surviving parental) were observed on day 7 and three times per week through day 21. At test termination body length and parental body mass (dry weight) were reported. For chemical analysis (LC/MS/MS) of S-2188 in test media duplicate samples were taken on days 0, 7, 14 and 21 from each test concentration. Measurements of pH, dissolved oxygen and temperature were made at initiation and once weekly in all vessels of each treatment and once daily on a rotating basis in a single representative vessel of each treatment.
Statistics:	In general data for parent mobility were acrisine transformed before further evaluation. Variance homogeneity and normal distribution were analysed by Bartletts test. If control groups can be pooled a t-Test was performed. All NOEC were derived by comparing each treatment group with

pooled controls:
Reproduction and mean dry weight: Williams's Test
Parent mortality and mean total body length: Wilcoxon's Rank Sum Test and Bonferroni's Test
Statistical analyses considering nominal concentrations are based on data of pooled controls. Statistical analyses considering mean measured concentrations are using only solvent control.

Findings:

Analytical data: The mean measured concentrations ranged from 85 – 95% of nominal concentrations.

Biological observation: First brood release by daphnids exposed to the control, 0.085, 0.18, 0.34, 0.76 and 1.4 mg a.s./L treatment levels occurred on test day 7, 7, 7, 7, 9 and 10.

Effects: See Table

Table 37: Summary of effects of long-term exposure of fenpyrazamine on *Daphnia magna*

Fenpyrazamine [mg a.s./L] (nom)	**S-2188 techn. [mg a.s./L] (mm)**	**Mean parent mortality at day 21 [%]**	**Offspring per surviving female at day 21**	**Mean dry weight of parent after 21 d [mg]**	**Mean body length of parent after 21 d [mm]**
Control	Control	10 ± 8	201 ± 14	1.60 ± 0.14	5.2 ± 0.05
Solvent control	Solvent control	7 ± 10	210 ± 29	1.74 ± 0.13	5.3 ± 0.08
Pooled control [a]	Pooled control [a]	9 ± 8	205 ± 22	1.67 ± 0.14	5.3 ± 0.07
0.1	0.085	5 ± 10	216 ± 22	1.57 ± 0.18	5.3 ± 0.03
0.2	0.18	10 ± 8	200 ± 16	0.67 ± 0.06	5.2 ± 0.04
0.4	0.34	2 ± 5	205 ± 13	1.66 ± 0.07	5.2 ± 0.02
0.8	0.76	0	156 ± 3*	1.49 ± 0.09*	4.8 ± 0.03**
1.6	1.4	7 ± 10	58 ± 24*	0.82 ± 0.22*	4.0 ± 0.31**
NOEC (based on mean measured)		1.4 mg a.s./L	0.34 mg a.s./L	0.34 mg a.s./L	0.34 mg a.s./L
LOEC (based on mean measured)		> 1.4 mg a.s./L	0.76 mg a.s./L	0.76 mg a.s./L	0.76 mg a.s./L
EC_{50} (based on mean measured)		> 1.4 mg a.s./L	1.1 mg a.s./L	n.d.	n.d.
MATC (based on mean measured)		0.51 mg a.s./L			

n.d…not determined, mm…mean measured, nom…nominal
[a] No statistically significant difference between control and solvent control.
* Significantly reduced compared to the pooled control, based on Williams' Test
** Significantly reduced compared to the pooled control, based on Wilcoxon's Rank Sum Test

Conclusion:

NOEC = 1.4 mg a.s./L (adult mortality)
LOEC > 1.4 mg a.s./L
NOEC = 0.34 mg a.s./L (reproduction)
LOEC = 0.76 mg a.s./L
NOEC = 0.34 mg a.s./L (growth, weight and length)
LOEC = 0.76 mg a.s./L
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.): Life-Cycle Test with Mysids (*Americamysis bahia*) Following Draft OPPTS Guideline 850.1350**
Author(s), year:	Lee, M.R., 2010b
Report/Doc. number:	Report No. QNW-0049, Study No. 12709.6293
Guideline(s):	OPPTS 850.1350
GLP:	Yes
Deviations:	The protocol states that temperature of the test solutions will be maintained at 25 ± 2°C. On test day 11, temperature readings exceeded this range with a maximum temperature recording of 29.7°C. Immediate action was taken to reduce the water bath temperature to the desired range. Test temperature was within the recommended range on the days prior to and following this excursion. Control survival and female reproduction met and exceeded guideline criteria and this temperature range is well within the tolerated range for this species. This deviation did not have a negative impact on the results or the interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	*Americamysis bahia* (≤ 23 h old)
Number of organisms:	F_0 life-cycle exposure initiation: 30 mysids per replicate, 2 replicates per treatment level and control F_0 life-cycle mysid pairing: 10 male/female pairs per replicate (approximately at day 12) F_1 generation: 10 per replicate, 2 replicates per treatment level and control
Type of test, duration:	Flow-through test, 28 d
Applied concentrations:	
Nominal:	0 (control), 0.0038, 0.0075, 0.015, 0.030, 0.060 and 0.120 mg a.s./L
Measured (mean):	- (control), 0.0032, 0.0068, 0.013, 0.024, 0.047 and 0.098 mg a.s./L
Test conditions:	
Water quality:	Natural seawater (filtered), salinity: 20 – 22‰
Temperature	26 - 30 °C
pH	7.5 – 8.1
O_2 content:	4.8 – 6.6 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light and 8 hours darkness, light intensity: 620 – 900 lux, 30 min transition period
Feeding	Mysids were fed with life brine shrimp (*Artemia salina*) naupilii (< 48 h old), twice daily. At least once a day food for was enriched with saturated fatty acids.
Test parameters:	F_0 generation (daily observations): Number of dead and living organisms, abnormal behaviour or appearance, number of offspring per female F_0 generation (at termination of the test): total body length and dry body weight. F_1 generation: Daily observations of stress abnormal behaviour (including discoloration, immobilization and inability to maintain position in the water column), and survival were made. Temperature, dissolved oxygen concentration, pH and salinity were

measured in each replicate on day 0 and alternated between replicates daily thereafter throughout the exposure period, for each treatment level and the control. Exposure solution temperature was continuously monitored in one control vessel.
Analytical measurements (HPLC/UV): During the in-life phase samples were removed from alternating replicate solutions of each treatment level and the control on days 0, 7, 14, 21 and 28.

Statistics: Normal distribution were analysed by Shapiro-Wilk's Test. As a check on the assumption of variance homogeneity, data of each endpoint were analysed using Bartlett's Test or Modified Levene's Test.
Since male and female survival data did not meet the assumption of homogeneity of variance, a non-parametric statistical procedure (Fisher's Exact Test with Bonferroni Holm adjustment) was used to evaluate survival data.
Mysid survival, growth and reproduction were analysed using William's Test.
CETIS^{TM} was used to perform the statistical computations.

Findings:

Analytical data: The mean measured concentrations ranged from 79 – 91% of nominal concentrations.

Survival: Following 28 d of exposure mean survival of 79 – 90 (male and female) was observed among mysids exposed to the treatment levels. Statistical analyses determined no significant difference in survival among organisms exposed to any of the treatment levels tested compared to the control data.
Since no concentration tested resulted in ≥ 50% reduction in survival, the 28 d LC_{50} value was empirically estimated to be > 0.098 mg a.s./L, the highest mean measured concentration tested.

Table 38: Summary of first generation (F_0) survival at termination of the 28 d life-cycle exposure

Fenpyrazamine [mg a.s./L] (mean measured)	28 d survival [%] (mean)	Post-pairing male survival [%] (mean)	Post-pairing female survival [%] (mean)
Control	80	76	85
0.0032	86	92	88
0.0068	81	79	93
0.013	90	79	100
0.024	79	73	93
0.047	82	88	83
0.098	87	88	95

Reproduction: At test termination the mean number of offspring per female was between 9.5 and 15 among mysids exposed to the treatment levels. Statistical analysis determined a significant difference in the mean number of offspring per female among organisms exposed to the 0.0032 mg a.s./L treatment level, compared to the control. However, due to the lack of a response in higher treatment levels, the reductions

observed at the 0.0032 mg a.s./L treatment level were not considered to be toxicant-related.

Table 39: Summary of first generation (F_0) reproductive success (offspring/female) at termination of the 28 d life-cycle exposure

Fenpyrazamine [mg a.s./L] (mean measured)	Females producing young [%] (mean)	Average number of offspring per female	Reproductive success (Average number of offspring/female/reproductive day)
Control	94	14	0.96
0.0032	95	9.5 *[a]	0.65
0.0068	100	15	0.98
0.013	100	13	0.86
0.024	94	12	0.84
0.047	100	13	0.86
0.098	95	13	0.92

* Significantly reduced compared to the control, based on Williams' Test.
[a] Due to the lack of dose response in higher treatment levels, this was not considered to be toxicant-related, nor biologically relevant.

Reproduction: The average total body length of male and female mysids was between 6.6 and 7.2 mm and 6.8 and 7.2 mm, respectively. Statistical analysis determined a significant difference in the average total body length of female mysids among organisms exposed to 0.0032, 0.047 and 0.098 mg a.s./L treatment levels, compared to the control.

Table 40: Summary of mean total body length and dry body weight of first generation (F_0) at termination of the 28 d life-cycle exposure

S-2188 [mg a.s./L] (mean measured)	Mean total body length [mm]		Dry body weight [mg]	
	Male	Female	Male	Female
Control	6.9	7.2	0.78	1.2
0.0032	6.8	6.9 *[a]	0.83	1.2
0.0068	7.0	7.2	0.87	1.2
0.013	7.2	7.2	0.83	1.1
0.024	6.8	7.1	0.78	1.1
0.047	6.9	6.8 *	0.79	1.1
0.098	6.6	7.0 *	0.83	1.1

* Significantly reduced compared to the control, based on Williams' Test.
[a] Due to the lack of dose response in the three treatment levels above 0.0032 mg a.s./L treatment level, this was not considered to be toxicant-related, nor biologically relevant.

Table 41: Summary of F_1 survival at 96 h post-release following exposure to fenpyrazamine

S-2188 [mg a.s./L] (mean measured)	96 h survival [%] (mean)
Control	100
0.0032	90
0.0068	100
0.013	95
0.024	100
0.047	100
0.098	100

Conclusion: LC_{50} > 0.098 mg a.s./L (F_0 and F_1 mortality)
NOEC = 0.098 mg a.s./L (F_0 and F_1 mortality, reproductive success, male body length, dry body weight)
NOEC = 0.024 µg a.s./L (F_0 female body length)
LOEC = 0.047 mg a.s./L (F_0 female body length)
MATC = 0.034 mg a.s./L
based on mean measured concentrations

Comment RMS: The observed effect on reproduction (average number of offspring per female) and growth (mean female body length) at the test concentration of 0.0032 mg a.s./L was not considered for the NOEC determination because the effect did not establish a dose-response relationship.

5.4.3 Algae and aquatic plants

Reference:	**S-2188 Technical Grade – Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata***
Author(s), year:	Hoberg, J.R., 2006a
Report/Doc. number:	Report No. QNW-0004, Study No. 13048.6509
Guideline(s):	OECD Guideline 201, JMAFF No.12-Nousan-8147, Alga Growth Inhibition Test (2-7-7), US EPA OPPTS 850.5400, EC Guideline Annex V - Method C.3
GLP:	Yes
Deviations:	During the definitive test, the initial control solution pH was 6.8. The 72 h control pH was 8.6 and exceeded the initial value by 1.8 units instead of acceptable 1.5 units as stated in the protocol. This increase in solution pH is due to photosynthesis by the algae and cannot be controlled. The 72 h mean control cell density ($95.33 * 10^4$ cells/mL) exceeds the required 16 times increase form the initial density ($1.0 * 10^4$ cells/mL). Therefore, the growth of the algal population was not affected by the increase solution pH.
Validity:	Acceptable

Material and methods:

Test substance: Fenpyrazamine (S-2188) technical grade, purity: 94.7%, batch: 030-050914-1G

Test species: Green alga (*Pseudokirchneriella subcapitata)*

Number of organisms: 1×10^4 cells/mL; 4 replicates per treatment group, medium control and

	solvent control
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (medium control and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L
Measured (mean):	- (medium control and solvent control), 0.053, 0.11, 0.22, 0.43 and 0.90 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2), 0.1 mL/L
Test conditions:	
Water quality:	Algal Assay Procedure (AAP) medium (according to OECD guideline), total hardness: 15 mg/L as $NaHCO_3$
Temperature:	22 – 24 °C
pH:	6.7 – 7.0 (0 h), 6.9 – 9.2 (96 h)
Incubation:	Continuous illumination at 3900 to 4700 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis (LC/MS/MS method) of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation, after 72 h and at termination, light intensity was measured ad daily intervals and temperature was monitored continuously.
Statistics:	Comparison of medium and solvent control: t-Test No significant differences, both sets of control data were pooled for all parameter. Determination of EC_{50}: TOXSTAT® software Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test Determination of NOEC: Williams' Test and Kruskal-Wallis' Test
Findings:	
Analytical data:	Mean measured concentrations were in the range of 83 – 90% of nominal concentrations over the whole test duration.
Morphological effects:	After 96 h of exposure cells were observed to be bloated in the highest test concentration.
Biomass, growth rate and cell density:	See Table

Table 42: Effects of technical fenpyrazamine on the green alga *P. subcapitata*

Fenpyrazamine [mg/L] (mean measured)	Percent inhibition relative to the pooled control [%]		
	Biomass (0 – 72 h)	Growth rate (0 – 72)	Cell density (0 – 96 h)
0 (pooled control) [a]	-	-	-
0.053	- 10	- 4	18
0.11	- 10	4	24 *
0.22	- 7	- 2	59 *
0.43	49*	20*	82 *
0.90	83*	39*	95 *
NOEC	0.22 mg a.s./L	0.22 mg a.s./L	0.053 mg a.s./L
EC_{50}	0.42 mg a.s./L	> 0.90 mg a.s./L	0.19 mg a.s./L

Fenpyrazamine [mg/L] (mean measured)	Percent inhibition relative to the pooled control [%]		
	Biomass (0 – 72 h)	Growth rate (0 – 72)	Cell density (0 – 96 h)
(95 % C.l.)	(0.40 – 0.46 mg a.s./L)		(0.15 – 0.22 mg a.s./L)

[a] Results of statistical analyses of control groups indicated no significant differences, thus pooled control are used for statistical analysis.
* Significantly different compared to the pooled control, based on Williams' Test

.

Conclusion: 72 h E_bC_{50} = 0.42 mg a.s./L
72 h E_rC_{50} > 0.90 mg a.s./L
96 h EC_{50} = 0.19 mg a.s./L (cell density)
72 h NOEC = 0.22 mg a.s./L (biomass and growth rate)
96 h NOEC = 0.053 mg a.s./L (cell density)
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Freshwater Blue-Green Alga, *Anabaena flos-aquae*, Following OPPTS Draft Guideline 850.5400**
Author(s), year:	Softcheck, K. A., 2010c
Report/Doc. number:	Report No. QNW-0058, Study No. 12709.6306
Guideline(s):	US EPA OPPTS 850.5400
GLP:	Yes
Deviations:	None
Validity:	The results of the study are used as additional information because of the high variance of cell densities within the replicates.

Material and methods:

Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Blue-green algae (*Anabaena flos-aquae)*
Number of organisms:	1 x 10^4 cells/mL; 3 replicates per treatment level and medium control.
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.051, 0.13, 0.32, 0.80, 2.0 and 5.0 mg a.s./L
Measured (mean):	- (medium control), 0.045, 0.12, 0.28, 0.74, 1.8 and 4.6 mg a.s./L
Solvent:	None
Test conditions:	
Water quality:	Algal Assay Procedure (AAP) medium (according to guideline)
Temperature:	23 - 24 °C
pH:	7.0 – 7.4 (0 h), 7.4 – 7.9 (96 h)
Conductivity:	97 – 100 µS/cm (0 h), 91 – 94 µS/cm (96 h)
Incubation:	Continuous illumination at 1600 – 2700 lux
Test parameters:	Cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope on each study day. For chemical analysis of test the substance, samples of test solution were taken at test initiation, after 72 h and at test termination. Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured ad daily intervals and temperature was monitored continuously.
Statistics:	Determination of EC_{50}: EC_{50} values were calculated for cell density by linear interpolation of response (percent reduction of cell density as

compared with the control) versus mean measured concentration.
Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test
Determination of NOEC: Williams' Test

Findings:

Analytical data: Mean measured concentrations were in the range of 88 – 93 % of nominal concentrations over the whole test duration.

Cell density: See Table

Table 43: Effects (cell density) of fenpyrazamine on the green alga *Anabaena flos-aquae*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition relative to the control [%] (0 – 96 h)**
Control	-
0.045	39
0.12	- 12
0.28	27
0.74	34
1.8	66
4.6	87 *
NOEC	0.74 mg a.s./L [a]
EC_{50} (95 % C.l.)	1.2 mg a.s./L (0.21 – 2.5 mg a.s./L)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Williams' Test
[a] The 96-hour NOEC was determined to be 1.8 mg a.s./L, based on Williams' Test. However, since 66% inhibition was observed at this treatment level, a more conservative estimate of the NOEC is 0.74 mg a.s./L, empirically estimated based on the dose response curve.

Conclusion: 96 h EC_{50} = 1.2 mg a.s./L
96 h NOEC = 0.74 mg a.s./L
based on mean measured concentrations

Comment RMS: According to the US EPA guideline the calculation of an EC_{50} value for growth rate is not required. The only endpoint derived from the study is based on cell counts. However, for classification the growth rate EC_{50} value should be considered. Hence, the RMS has re-calculated the EC_{50} values using ToxRat® Software.

Statistical analyses of growth rate:
Determination of EC_{50} values: Probit analysis using simple linear regression
Testing of normal distribution: Shapiro-Wilk's Test
Testing of variance homogeneity: Levene's Test
Determination of NOEC: Williams Multiple Sequential t-Test

Based on the statistical analyses the following results were determined.

Table 44: Effects of fenpyrazamine on the green alga *Navicula pelliculosa*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition relative to the control [%] (0 – 96 h)**		
	Cell counts	**Growth rate**	**Yield**
Control	-	-	-
0.045	39	12	39
0.12	-12	- 1	-12

Fenpyrazamine [mg/L] (mean measured)	Inhibition relative to the control [%] (0 – 96 h)		
	Cell counts	Growth rate	Yield
0.28	27	7	28
0.74	34	8	34
1.8	66	24*	67
4.6	87*	52*	88*
NOEC	0.74 mg a.s./L [a]	0.74 mg a.s./L	0.74 mg a.s./L [b]
96 h EC_{50} (95 % C.l.)	1.221 mg a.s./L (n.d.)	9.442 mg a.s./L (n.d.)	1.173 mg a.s./L (n.d.)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Williams' Test
[a] The 96-hour NOEC was determined to be 1.8 mg a.s./L, based on Williams' Test. However, since 66% inhibition was observed at this treatment level, a more conservative estimate of the NOEC is 0.74 mg a.s./L, empirically estimated based on the dose response curve.
[b] The 96-hour NOEC was determined to be 1.8 mg a.s./L, based on Williams' Test. However, since 67% inhibition was observed at this treatment level, a more conservative estimate of the NOEC is 0.74 mg a.s./L, empirically estimated based on the dose response curve.

Conclusion: 96 h E_rC_{50} = 9.442 mg a.s./L (growth rate)
96 h NOEC = 0.74 (growth rate)
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Freshwater Diatom, *Navicula Pelliculosa*, Following OPPTS Draft Guideline 850.5400**
Author(s), year:	Softcheck, K. A., 2010b
Report/Doc. number:	Report No. QNW-0057, Study No. 12709.6304
Guideline(s):	US EPA OPPTS 850.5400
GLP:	Yes
Deviations:	None
Validity:	Acceptable

Material and methods:

Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Diatom algae (*Navicula pelliculosa)*
Number of organisms:	1 x 10^4 cells/mL; 4 replicates per treatment level and medium control.
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.0051, 0.013, 0.032, 0.080, 0.2 and 0.5 mg a.s./L
Measured (mean):	- (medium control), 0.0049, 0.012, 0.030, 0.074, 0.19 and 0.47 mg a.s./L
Solvent:	None
Test conditions:	
Water quality:	Algal Assay Procedure (AAP) medium (according to guideline)
Temperature:	23 - 24 °C
pH:	7.2 – 7.6 (0 h), 7.4 – 8.7 (96 h)
Conductivity:	270 µS/cm (0 h), 270 - 280 µS/cm (96 h)
Incubation:	Continuous illumination at 3900 - 4700 lux
Test parameters:	At each subsequent 24-hour interval, cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope at each 24-hour interval.

Algistatic/algicidal properties: A sample was removed from the composite of the four replicate vessels of the 0.50 and 0.080 mg a.s./L nominal test concentrations at test termination. The samples were then diluted with freshly prepared AAP medium to prepare two subcultures with nominal concentrations of 0.0051 mg a.si./L. The performance of the subcultures was used to determine if the effects of the test substance on the alga were algistatic, in which case cells would resume growth in the subculture, or algicidal, in which case no growth would occur in the subculture. The
subcultures were incubated for up to nine days under conditions consistent with those maintained during the definitive exposure. During this period, the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed.
For chemical analysis of test the substance, samples of test solution were taken at test initiation and at test termination.
Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.

Statistics: Determination of EC_{50}: EC_{50} values were calculated for cell density by linear interpolation of response (percent reduction of cell density as compared with the control) versus mean measured concentration.
Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test
Determination of NOEC: Williams' Test

Findings:

Analytical data: Mean measured concentrations were in the range of 92 – 96 % of nominal concentrations over the whole test duration.

Biological data: The observations indicate that the test substance has an algistatic, rather than algicidal effect, on the growth of *N. pelliculosa* at 0.080 and 0.50 mg a.s./L.
All cells exposed to S-2188 appeared normal compared to the control cells.

Cell density: See Table

Table 45: Effects (cell density) of fenpyrazamine on the green alga *Navicula pelliculosa*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition relative to the control [%] (0 – 96 h)**
Control	-
0.0049	- 11
0.012	55*
0.030	75*
0.074	81*
0.19	99*
0.47	99*
NOEC	0.0049 mg a.s./L
96 h EC_{50} (95 % C.l.)	0.011 mg a.s./L (0.010 – 0.012 mg a.s./L)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Williams' Test

Conclusion: 96 h EC_{50} = 0.011 mg a.s./L
96 h NOEC = 0.0049 mg a.s./L
based on mean measured concentrations

Comment RMS: According to the US EPA guideline the calculation of an EC_{50} value for growth rate is not required. The only endpoint derived from the study is based on cell counts. However, for classification the growth rate EC_{50} value should be considered. Hence, the RMS has re-calculated the EC_{50} values using ToxRat® Software.

Statistical analyses of growth rate:
Determination of EC_{50} values: Probit analysis simple (weighed) linear regression
Testing of normal distribution: Shapiro-Wilk's Test
Testing of variance homogeneity: Levene's Test
Determination of NOEC: Williams Multiple Sequential t-Test

Based on the statistical analyses the following results were determined.

Table 46: Effects of fenpyrazamine on the green alga *Navicula pelliculosa*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition relative to the control [%] (0 – 96 h)**		
	Cell counts	**Growth rate**	**Yield**
Control	-	-	-
0.0049	- 11	- 2	-11
0.012	55*	17	55*
0.030	75*	29	75*
0.074	81*	36	82*
0.19	99*	141*	100*
0.47	99*	107*	100*
NOEC	0.0049 mg a.s./L	0.074 mg a.s./L	0.0049 mg a.s./L
96 h EC_{50} (95 % C.l.)	0.009 mg a.s./L (0.004 – 0.13 mg a.s./L)	0.202 mg a.s./L (0.134 – 0.405 mg a.s./L)	0.008 mg a.s./L (0.005 – 0.012 mg a.s./L)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Williams' Test

Conclusion: 96 h E_rC_{50} = 0.202 mg a.s./L (growth rate)
96 h NOEC = 0.074 mg a.s./L (growth rate)
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*, Following OPPTS Draft Guideline 850.5400**
Author(s), year:	Softcheck, K. A., 2010a
Report/Doc. number:	Report No. QNW-0056, Study No. 12709.6307
Guideline(s):	US EPA OPPTS 850.5400
GLP:	Yes
Deviations:	The protocol states that the test vessels will be illuminated to a light intensity of 3900 to 4700 lux (360 to 440 footcandles). On test day 3, the actual light intensity over the test area ranged from 330 to 470 footcandles. Since the actual range was only slightly outside of the range required by the protocol, this deviation did not impact the results or interpretation of this study.
Validity:	Acceptable

Material and methods:

Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Marine diatom algae (*Skeletonema costatum)*
Number of organisms:	7.7 x 10^4 cells/mL; 3 replicates per treatment level and medium control.
Type of test, duration:	Static test, 96 hours
Applied concentrations:	
Nominal:	0 (medium control), 0.0040, 0.010, 0.025, 0.063, 0.16, 0.40 and 0.98 mg a.s./L
Measured (mean):	- (medium control), 0.0045, 0.011, 0.028, 0.075, 0.18, 0.42 and 1.2 mg a.s./L
Solvent:	None
Test conditions:	
Water quality:	Artificial Enriched Seawater (AES) medium (according to guideline)
Temperature:	19 -20 °C
pH:	7.8 – 8.2 (0 h), 7.6 – 8.5 (96 h)
Conductivity:	48000 – 49000 µS/cm (0 h), 50000 – 51000 µS/cm (96 h)
Incubation:	14 hours light:10 hours darkness at 3500 - 5000 lux
Test parameters:	At each subsequent 24-hour interval, cell counts were estimated using a haemocytometer and microscope. Observations of the health and morphology of the algal cells were made under the microscope at each 24-hour interval. Algistatic/algicidal properties: A sample was removed from the composite of the most inhibited test concentrations (0.98 mg a.s./L) at test termination. The sample was then diluted with freshly prepared AES medium to prepare subcultures with a nominal concentration of 0.0040 mg a.s./L. The performance of the subcultures were used to determine if the effects of the test substance on the algae were algistatic, in which case cells would resume growth in the subculture, or algicidal, in which case no growth would occur in the subculture. The subcultures were incubated for up to ten days under conditions consistent with those maintained during the definitive exposure. During this period, the subcultures were microscopically examined every other day to determine whether or not cell growth had resumed. For chemical analysis of test the substance, samples of test solution

were taken at test initiation and at test termination.
Measurements of pH and conductivity were made at initiation and at termination, light intensity was measured at daily intervals and temperature was monitored continuously.

Statistics: Determination of EC_{50}: EC_{50} values were calculated for cell density by linear interpolation of response (percent reduction of cell density as compared with the control) versus mean measured concentration.
Normal distribution and homogeneity of variance: Shapiro Wilks' Test and Bartlett's Test
Determination of NOEC: Williams' Test

Findings:

Analytical data: Mean measured concentrations were in the range of 110 - 120 % of nominal concentrations over the whole test duration.

Biological data: The observations indicate that the test substance has an algistatic, rather than algicidal effect on the growth of *Skeletonema costatum* at 0.98 mg a.s./L.

Cell density: See Table

Table 47: Effects of fenpyrazamine on the green alga *Skeletonema costatum*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition of cell density relative to the control [%] (0 – 96 h)**
Control	-
0.0045	21*
0.011	3
0.028	54*
0.075	76*
0.18	90*
0.42	95*
1.2	97*
NOEC	0.011 mg a.s./L
96 h EC_{50} (95 % C.l.)	0.027 mg a.s./L (0.024 – 0.035 mg a.s./L)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Dunnett's Test

Conclusion: 96 h EC_{50} = 0.027 mg a.s./L
96 h NOEC = 0.011 mg a.s./L
based on mean measured concentrations

Comment RMS: According to the US EPA guideline the calculation of an EC_{50} value for growth rate is not required. The only endpoint derived from the study is based on cell counts. However, for classification the growth rate EC_{50} value should be considered. Hence, the RMS has re-calculated the EC_{50} values using ToxRat® Software.

Statistical analyses of growth rate:
Determination of EC_{50} values: Probit analysis using simple linear regression
Testing of normal distribution: Shapiro-Wilk's Test
Testing of variance homogeneity: Levene's Test

Determination of NOEC: Williams Multiple Sequential t-Test (cell counts, yield) and Welch-t Test for inhomogeneous variances with Bonferroni-Holm Adjustment (growth rate)

Based on the statistical analyses the following results were determined.

Table 48: Effects of fenpyrazamine on the green alga *Skeletonema costatum*

Fenpyrazamine [mg/L] (mean measured)	**Inhibition relative to the control [%] (0 – 96 h)**		
	Cell counts	**Growth rate**	**Yield**
Control	-	-	-
0.0045	21*	10	23*
0.011	3*	1	3*
0.028	54*	34**	60*
0.075	76*	63**	85*
0.18	90*	99**	100*
0.42	95*	127**	105*
1.2	97*	161**	108*
NOEC	0.011 mg a.s./L	0.011 mg a.s./L	0.011 mg a.s./L
96 h EC_{50} (95 % C.l.)	0.038 mg a.s./L (0.009 – 0.099 mg a.s./L)	0.034 mg a.s./L (0.013 – 0.068 mg a.s./L)	0.022 mg a.s./L (0.006 – 0.046 mg a.s./L)

Negative value indicates an increase of algal growth
* Significantly different compared to the control, based on Williams' Test
** Significantly different compared to the control, based on Welch-t Test with Bonferroni-Holm Adjustment

The observed effects (cell counts, yield) at the test concentration of 0.0045 mg a.s./L was not considered for the NOEC determination because the effect did not establish a dose-response relationship. Hence, the NOEC was determined to be 0.011 mg a.s./L.

Conclusion: 96 h E_rC_{50} = 0.034 mg a.s./L (growth rate)
96 h NOEC = 0.011 (growth rate)
based on mean measured concentrations

Reference:	**V-10135 T.G. (S-2188) 7-Day Toxicity Test with Duckweed (*Lemna gibba*) Following OPPTS Draft Guideline 850.4400**
Author(s), year:	Softcheck, K. A., 2010d
Report/Doc. number:	Report No. QNW-0055, Study No. 12709.6305
Guideline(s):	US EPA OPPTS 850.4400
GLP:	Yes
Deviations:	The protocol states that the pH will be measured in freshly prepared solutions at test initiation, at the beginning of each renewal period, at the end of each renewal period and at test termination. On day 3 of the definitive exposure, the freshly prepared test solution at the highest treatment level (5.0 mg a.s./L) was inadvertently discarded prior to pH measurement. Since the 5.0 mg a.s./L test solution prepared on test day 3 was made using the same procedures as at the other renewal periods (day 0 and day 5), this deviation is considered to have had no impact on the results or interpretation of this study.
Validity:	Acceptable

Material and methods:	
Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Duckweed (*Lemna gibba*)
Number of organisms:	12 fronds (plants with 3 to 4 fronds each), dry weight: 1.3 mg; 3 replicates per treatment level and medium control.
Type of test, duration:	Static-renewal test, 7 days On test day 3 and 5, fronds were transferred to newly prepared test solutions.
Applied concentrations:	
Nominal:	0 (medium control), 0.02, 0.051, 0.14, 0.32, 0.80, 2.0 and 5.0 mg a.s./L
Measured (mean):	- (medium control), 0.023, 0.06, 0.15, 0.36, 0.91, 2.3 and 5.9 mg a.s./L
Solvent:	None
Test conditions:	
Water quality:	20X Algal Assay Procedure (AAP) medium (according to guideline)
Temperature:	23 – 24 °C
pH:	7.7 – 8.1 (new medium), 8.3 – 8.8 (aged medium)
Incubation:	Continuous lightening at 5000 - 6500 lux
Test parameters:	On days 3, 5 and at test termination (day 7), fronds were counted and observations were made. At test termination, after frond density determination was complete, the dry weight was measured. At the beginning and end of the longest (three days) renewal period (days 0 and 3), a single algal medium sample was removed from each test concentration and the control and analysed for V-10135 concentration. The pH of the exposure solutions was measured at test initiation (new solutions), in each aged and new solutions at each renewal period, and at test termination (aged solutions). Light intensity was measured at daily intervals and temperature was monitored continuously.
Statistics:	Determination of EC_{50}: The EC50 values were calculated, when possible, for frond densities, average growth rate (based on frond density) and dry weight biomass at test termination by linear

interpolation of response percent reduction of frond density, growth rate or biomass as compared with the control) versus mean measured concentration.
Frond number, biomass, growth rate: Based on the results of Shapiro-Wilks' and Bartlett's Tests, this data set passed the requirements for normality and homogeneity of variance, therefore, Williams' Test was used to determine treatment-related effects.

Findings:

Analytical data: Mean measured concentrations were in the range of 110 - 120 % of nominal concentrations over the whole test duration.

Biological data: At the test concentrations of 0.91 mg a.s./L the fronds were observed to be smaller than the control fronds. At the two highest test concentrations (2.3 and 5.9 mg a.s./L) this effect (smaller fronds) was observed too. Additionally, the plants show a less root formation than the control plants.

Frond number, biomass, growth rate: See Table

Table 49: Effects of fenpyrazamine on the duckweed *Lemna gibba*

Fenpyrazamine [mg/L] (mean measured)	Percent inhibition relative to the control [%]		
	Mean frond number	Mean frond dry weight (biomass)	Mean growth rate [a] (0 – 7 d)
Control	-	-	-
0.023	- 3	6	- 2
0.060	- 11	1	- 5
0.15	3	12*	0
0.36	10*	22*	2
0.91	34*	45*	14*
2.3	64*	73*	36*
5.9	79*	90*	55*
NOEC	0.15 mg a.s./L	0.060 mg a.s./L	0.36 mg a.s./L
96 h EC_{50} (95 % C.l.)	1.5 mg a.s./L (1.4 – 1.7 mg a.s./L)	1.2 mg a.s./L (1.0 – 1.3 mg a.s./L)	4.9 mg a.s./L (4.5 – 5.8 mg a.s./L)

Negative value indicates an increase of growth
* Significantly different compared to the control, based on Williams's Test
[a] Based on frond density

Conclusion:

7 d EC_{50} = 1.5 mg a.s./L (frond number)
7 d EC_{50} = 1.2 mg a.s./L (biomass)
7 d EC_{50} = 4.9 mg a.s./L (growth rate)
7 d NOEC = 0.15 mg a.s./L (frond number)
7 d NOEC = 0.06 mg a.s./L (biomass)
7 d NOEC = 0.36 mg a.s./L (growth rate)
based on mean measured concentrations

5.4.4 Other aquatic organisms (including sediment)

Reference:	**V-10135 T.G. (S-2188 T.G.) Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions, Following OPPTS Guideline (Draft) 850.1025**
Author(s), year:	York, D. O., 2010
Report/Doc. number:	Report No. QNW-0046, Study No. 12709.6302
Guideline(s):	U.S. EPA OPPTS 850.1025, US EPA Guideline – Ecological Effects
GLP:	Yes
Deviations:	The protocol states that total dissolved oxygen will not be allowed to drop below 60% of saturation during the test. At the 24-hour interval, dissolved oxygen levels in replicate A of the 0.50 mg a.s./L nominal treatment level and replicate B of the 0.25 mg a.s./L nominal treatment level were 50 and 56%, respectively. All test vessels were scraped and siphoned and aeration was initiated in all test vessels. Dissolved oxygen levels in these replicates were rechecked later in the day and found to be above 60% of saturation. The deviation was of short duration and the dissolved oxygen levels did not drop to levels that are considered detrimental to oysters; therefore, this deviation did not have a negative impact on the results or interpretation of this study.
Validity:	Acceptable

Material and methods:	
Test substance:	V-10135 (S-2188) technical grade, purity: 98.8%, batch: AS 2177c
Test species:	Eastern oysters (*Crassostrea virginica*)
Number of organisms:	2 replicates each with 20 oysters per treatment, control and solvent control.
Age:	Oysters were of similar age (reproductively immature, no gametes stored), mean valve height of 35 ± 3.1 mm (n = 30) Prior to testing, 3 to 5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge using a fine-grit grinding wheel.
Type of test, duration:	Flow-through test, 96 hours
Applied concentrations:	
Nominal:	0 (control and solvent control), 0.063, 0.13, 0.25, 0.50 and 1.0 mg a.s./L
Measured (mean):	- (control and solvent control), 0.066, 0.12. 0.24, 0.49 and 1.0 mg a.s./L
Solvent:	Dimethylformamide (DMF, CAS No. 68-12-2)
Test conditions:	
Water quality:	Natural seawater (filtered), salinity: 21 – 22 ‰
Temperature:	20 – 21 °C
pH:	7.4 – 7.9 (0 - 96 h)
O_2 content:	4.1 – 7.9 mg O_2/L (> 60 % saturation)
Light regime:	16 hours light / 8 hours darkness, light intensity: 240 - 1100 lux
Feeding:	During the exposure, the oysters received supplemental feedings (3 times daily) of algae (*Tetraselmus maculata*), approximately 10^5 cells/mL per test aquarium.
Test parameters:	Biological observations (e.g. visible abnormalities, such as excessive mucous production or a failure to siphon and feed, as evidenced by a lack of faecal and pseudofaecal production) and observation of the physical characteristic of the test solutions were made at exposure

initiation, at the 6-hour interval and at each 24-hour interval until termination of the test. Sublethal effects were determined by a comparison of the performance and appearance of the exposed oysters to that of the control oysters. After 96 hours of exposure, the oysters were removed from the test aquaria and the new shell growth was measured microscopically.
Measurements of pH, temperature, salinity and dissolved oxygen concentrations were made at initiation and once daily.
For chemical analysis of S-2188 in the test media samples were taken at test initiation (0 h) and termination (96 h).

Statistics: Significance between control and solvent control: t-Test
EC_{50}: Dunnett's Test, NOEC: Directly from the raw data

Findings:

Analytical data: The overall mean measured concentration ranged from 93 - 110 % of nominal concentrations.

Effects: No mortality or adverse effects were observed among oysters at any of the treatment levels. Following 96 hours of exposure 30, 21, 21, 32 and 75 % reduction in shell growth was observed in test concentrations 0.066, 0.12, 0.24, 0.49 and 1.0 mg/L, respectively.
See Table

Table 50: Effects on oysters (*Crassostrea virginica*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./L] (mean measured)	Mean shell deposition [mm] after 96 hr (SD) [a]	Mean reduction [%] compared to the pooled control
Control	2.3 (1.0)	-
Solvent control	2.3 (0.9)	-
Pooled control	2.3 (0.9)	-
0.066	1.6 (0.7)	30
0.12	1.8 (0.8)	21
0.24	1.8 (0.9)	21
0.49	1.6 (0.8)	32 *
1.0	0.6 (0.5)	75 *
NOEC = 0.24 mg a.s./L		
EC_{50} (96 h) = 0.66 mg a.s./L (95 % C.I. 0.0 – 2.6 mg a.s./L)		

SD…Standard deviation
* Statistically significant compared to the pooled control, based on Dunnett's Test

Conclusion: 96 h EC_{50} = 0.66 mg a.s./L
96 h NOEC = 0.24 mg a.s./L
based on mean measured concentrations

Reference:	**42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to V-10135 (S-2188) Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods**
Author(s), year:	Picard, C. R., 2010a
Report/Doc. number:	Report No. QNW-0052, Study No. 12709.6290
Guideline(s):	U.S. EPA OPPTS 850.1735, US EPA Guideline – Ecological Effects, EPA Test Method 100.4, OECD 218
GLP:	Yes
Deviations:	The protocol states that the test will be conducted in a temperature-controlled water bath maintained at the appropriate test temperature of 23 ± 1 ºC. During this exposure, the temperature as measured by the minimum/maximum thermometer ranged from 21 to 25 ºC. Instantaneous measurements in some test vessels on test days 7, 15, 16, 20, 25, 26 and 27 exceeded the temperature range stated in the protocol, the maximum temperature being 25 ºC. Since these parameters are within the tolerance range for the test organisms, these deviations did not have a negative impact on the results or interpretation of the study. The protocol states that the overlying water source will have an approximate specific conductance range of 110 to 380 µmhos/cm. The range listed in the protocol does not accurately reflect the current characteristics of the dilution water. During this exposure, the overlying water source had a specific conductance range of 420 to 460 µmhos/cm. While fluctuations in the overlying water parameters are often caused by seasonality, the specific conductance of the source water observed in this study is within the typical range. Since these parameters are within the tolerance range for the test organisms, this deviation did not have a negative impact on the results or interpretation of the study.
Validity:	Acceptable

Material and methods:

Test substance:	Radiolabeled: [^{14}C] V-10135 ([^{14}C] S-2188) technical grade, purity: 99.2 %, batch: CFQ14368 Nonradiolabeled: V-10135 (S-2188) technical grade, purity: 98.8 %, batch: AS 2177c
Test species:	Freshwater amphipod (*Hyalella azteca*)
Number of organisms:	12 replicates each with 10 amphipods per treatment, control and solvent control. 3 additional replicates per treatment and controls for analytical measurements.
Age:	Juvenile amphipods, 8 days old
Type of test, duration:	Static-renewal test, 42 days
Applied concentrations:	
Nominal:	0 (control and solvent control), 6.3, 13, 25, 50 and 100 mg a.s./kg
Measured (mean):	- (control and solvent control), 5.6, 10, 24, 38 and 94 mg a.s./kg
Solvent:	Acetone (CAS No. 67-64-1)
Test conditions:	
Water quality:	Laboratory well water, total hardness of 64 - 84 mg/L as $CaCO_3$, total alkalinity of 18 - 28 mg/L as $CaCO_3$, specific conductivity of 390 – 470 µmhos/cm, Ammonia of ≤ 0.10 – 2.0 mg/L as N

Temperature:	22 – 25 °C
pH (water):	6.9 – 7.5
O_2 content:	4.8 – 8.3 mg O_2/L (> 60 % saturation)
Sediment quality:	Artificial sediment (according to OECD guideline 218)
Particle size distribution:	79% sand, 4% silt and 17% clay
Light regime:	16 hours light / 8 hours darkness, light intensity: 480 – 700 lux
Feeding:	During the exposure, the freshwater amphipods were fed with flaked fish food suspension (YCT) daily.
Test parameters:	Daily observations of organism behaviour were made. Survival and growth (dry weight) of amphipods in each of four randomly selected replicate vessels was determined on test day 28 by sieving the sediment to remove all surviving amphipods. The amphipods in the remaining eight replicates following determination of survival and growth were also removed by sieving and survival of these organisms was recorded. The surviving amphipods from these replicates were then placed in water-only exposure vessels. Reproduction and survival of the amphipods was measured on test days 35 and 42 by removing and counting the adults and offspring in each replicate beaker. In addition, any offspring observed at the end of the sediment exposure phase (test day 28) were counted and recorded. On day 35, adults were enumerated to assess day 35 survival and returned to their respective test vessels after reproduction had been assessed. At test termination (day 42), the adult amphipods were enumerated to assess day 42 survival and preserved in sugar formalin solution. The number of adult males and females were determined following preservation. Mature males are identified by the enlarged second gnathopod. Those amphipods not identified as males were recorded as female amphipods. Reproduction for both day 35 and 42 is expressed as the number of young per adult female amphipod in each test chamber based on the number of females present at test day 42. Dissolved oxygen concentration, temperature and pH were measured and recorded for each test vessel at test initiation (replicates A through L), test day 28 (replicates A through L), and in the remaining eight vessels set up for biological observations on test day 29 and at test termination (test day 42). Total hardness, alkalinity, specific conductance and total ammonia concentration were monitored in the overlying water at test initiation, test day 28, test day 29 and at test termination (test day 42). For chemical analysis of S-2188 in the test media samples were taken of overlaying water, pore water and sediment at day 0, 14 and 28.
Statistics:	Significance between control and solvent control: t-Test Normality: Chi-Square Test (survival, growth, reproduction, sex ratio) Homogeneity of variance: Bartlett's Test EC_{50}: Wilcoxon's Rank Sum Test with Bonferroni's Adjustment (survival, reproduction, day 28 and 35), Dunnett's Test (growth, day 28), Bonferroni's t-Test (survival, growth, reproduction and sex ratio, day 42) NOEC: Directly from the raw data

Findings:

Analytical data: The overall mean measured concentration in the sediment ranged from 77 - 95 % of nominal concentrations.

Effects: See Table

Table 51: Effects (survival, growth) on freshwater amphipods (*Hyalella azteca*) exposed to technical fenpyrazamine at day 28

Fenpyrazamine [mg a.s./kg] (mean measured)	Day 28	
	Mean percent survival (SD) [%]	Mean dry weight per amphipod (SD) [mg]
Control	96 (7)	0.29 (0.06)
Solvent control	97 (5)	0.21 (0.03)
5.6	93 (9)	0.30 (0.07)
10	87 (9)	0.30 (0.16)
24	77 (19) *	0.17 (0.02) [a]
38	77 (19) *	0.22 (0.04) [a]
94	26 (16) *	0.12 (0.02) [a]
NOEC	10 mg a.s./kg	10 mg a.s./kg
LC_{50}/EC_{50} (95% C.I.)	69 mg a.s./kg (62 – 76 mg a.s./kg)	67 mg a.s./kg (45 – 85 mg a.s./kg)

SD…Standard deviation
* Statistically significant compared to the control, based on Wilcoxon's Rank Sum Test.
[a] Treatment level was excluded from statistical analyses of growth and reproduction due to the survival effect observed.

Table 52: Effects (survival, growth, reproduction, sex ratio) on freshwater amphipods (*Hyalella azteca*) exposed to technical fenpyrazamine at day 42

Fenpyrazamine [mg a.s./kg] (mean measured)	Day 42			
	Mean percent survival (SD)	Mean dry weight per amphipod (SD) [mg]	Mean number of offspring per female (SD)	Mean Male:Female Ratio (SD)
Control	95 (8)	0.43 (0.05)	4.06 (2.20)	1.41 (0.69)
Solvent control	93 (7)	0.44 (0.07)	3.81 (2.16)	1.09 (0.55)
5.6	93 (9)	0.39 (0.09)	2.00 (1.91)	0.93 (0.74)
10	77 (20)	0.40 (0.10)	2.67 (2.09)	0.80 (0.43)
24	61 (30)*	0.31 (0.16) [a]	4.25 (4.17) [a]	0.72 (0.67) [a]
38	71 (22)*	0.37 (0.12) [a]	3.28 (3.26) [a]	0.91 (0.42) [a]
94	24 (15)*	0.41 (0.19) [a]	0.45 (0.45) [a]	0.25 (0.43) [a]
NOEC	10 mg a.s./kg	10 mg a.s./kg	10 mg a.s./kg	10 mg a.s./kg
LC_{50}/EC_{50} (95% C.I.)	61 mg a.s./kg (48 – 72 mg a.s./kg)	> 94 mg a.s./kg (n.a.)	55 mg a.s./kg (29 – 72 mg a.s./kg)	n.a.

SD…Standard deviation, n.a…not applicable
* Statistically significant compared to the control, based on Bonferroni's t-Test.
[a] Treatment level was excluded from statistical analyses of growth and reproduction due to the survival effect observed.

Conclusion: 42 d NOEC = 10 mg a.s./kg (mortality, sex ratio, growth, reproduction)
42 d LC_{50} = 61 mg a.s./kg (mortality)
42 d EC_{50} = 55 mg a.s./kg (reproduction)
42 d LOEC > 10 mg a.s./kg (sex ratio, growth, reproduction)
based on mean measured concentrations

Reference:	**V-10135 (S-2188) 28-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment Following EPA Test Methods**
Author(s), year:	Picard, C. R., 2010b
Report/Doc. number:	Report No. QNW-0053, Study No. 12709.6291
Guideline(s):	US EPA Guideline – Ecological Effects, Guideline Series 850.0000, Sediment Testing: Whole Sediment Chronic (Marine)
GLP:	Yes
Deviations:	The protocol states that the test will be conducted in a temperature controlled water bath maintained at the appropriate test temperature of 25 ± 1° C. During this exposure, the temperature, as measured by a minimum/maximum thermometer, exceeded the temperature range specified in the protocol on test days 20, 21, 23 and 25. On these test days, the maximum temperature observed was 27° C. Instantaneous measurements exceeded the temperature range stated in the protocol on test day 25; the maximum temperature observed being 27° C. These temperatures are within the tolerance range of the test organism; therefore, this deviation did not impact the results or the interpretation of this study. The protocol states that each exposure vessel will be fed three times a week following renewal of the overlying water. During this study, feeding in all exposure replicates was suspended on test day 7 due to minor fungal growth on the sediment surface in a few replicates. Since the feeding rates were kept consistent across all treatment levels and controls allowing for the negative control acceptability criteria to be met, this deviation did not significantly impact the outcome of the exposure.
Validity:	Acceptable

Material and methods:

Test substance:	Radiolabeled: [^{14}C] V-10135 ([^{14}C] S-2188) technical grade, purity: 99.2 %, batch: CFQ14368 Nonradiolabeled: V-10135 (S-2188) technical grade, purity: 98.8 %, batch: AS 2177c
Test species:	Estuarine amphipod (*Leptocheirus plumulosus*)
Number of organisms:	Ten replicates were maintained for each test concentration and controls. Five replicates were used to evaluate the biological response of the test organisms. The remaining five replicates were maintained for the purpose of chemical analysis and pore water quality measurements. Each replicate vessel contained 20 amphipods, a total of 100 amphipods per concentration or controls for the replicates maintained for monitoring the biological response. The additional replicates were maintained under the same conditions and contained test organisms, but were not used to evaluate the biological response of the test organisms.
Age:	Neonates, size: 0.25 – 0.60 mm
Type of test, duration:	Static-renewal test, 28 days
Applied concentrations:	
Nominal:	0 (control and solvent control), 6.3, 13, 25, 50 and 100 mg a.s./kg
Measured (mean):	- (control and solvent control), 3.0, 6.6, 12, 26 and 51 mg a.s./kg
Solvent:	Acetone (CAS No. 67-64-1)

Test conditions:	
Overlaying water quality:	Natural seawater (filtered), salinity: 20 – 22 ‰
Temperature:	25 – 27 °C
pH (water):	7.2 – 8.1
O_2 content:	5.8 – 7.8 mg O_2/L (> 60 % saturation)
Ammonium	≤ 1.0 mg/L as N (Day 0), 0.21 – 0.65 mg/L as N (Day 28)
Pore water quality:	Natural seawater (filtered), salinity: 20 – 24 ‰
Temperature:	22 – 24 °C
pH (water):	6.8 – 6.9
Ammonium	10 - 18 mg/L as N (Day 0), 4.5 – 13 mg/L as N (Day 28)
Sediment quality:	Natural marine sediment, organic carbon: 6.9 %, pH 7.4
Particle size distribution:	45% sand, 33% silt and 22% clay
Light regime:	16 hours light / 8 hours darkness, light intensity: 630 - 840 lux
Feeding:	The amphipods were fed a diet consisting of a flaked fish food suspension (10 mg/mL). During the exposure, food was added to each vessel three times per week, following renewal of the overlying water.
Test parameters:	All vessels were examined at test initiation and at 24-hour intervals thereafter, until test termination (day 28). Observations of mortality and abnormal behaviour were made. At test termination (day 28), the total number of surviving amphipods was determined in each test vessel by sieving the sediment to remove all surviving amphipods. Reproduction was determined as the number of young per surviving adult amphipod in each replicate vessel. Growth was also determined as body weight (dry) at test termination. Dissolved oxygen concentration, temperature, salinity and pH were measured daily. In addition, the temperature was continuously monitored. For chemical analysis of S-2188 in the test media samples were taken of overlaying water, pore water and sediment at day 0, 14 and 28.
Statistics:	Significance between control and solvent control: t-Test Normality: Chi-Square Test (survival, growth, reproduction) Homogeneity of variance: Bartlett's Test EC_{50}: Wilcoxon's Rank Sum Test with Bonferroni's Adjustment (survival), Bonferroni's t-Test (growth, reproduction) NOEC: Directly from the raw data
Findings:	
Analytical data:	The overall mean measured concentration in the sediment ranged from 48 – 51 % of nominal concentrations.
Effects:	See Table

Table 53: Effects (survival, growth) on marine amphipods (*Leptocheirus plumulosus*) exposed to technical fenpyrazamine

Fenpyrazamine [mg a.s./kg] (mean measured)	Day 28		
	Mean percent survival (SD)	Mean dry weight per amphipod (SD) [mg]	Average number of offspring per female (SD)
Control	84 (7)	2.64 (0.20)	16 (8)
Solvent control	79 (2)	2.36 (0.21)	10 (8)
3.0	74 (22)	2.00 (0.59)	8 (4)***
6.6	76 (15)	2.30 (0.21)	12 (2)
12	71 (15)	1.84 (0.56)**	7 (5)**
26	0 (0)*	n.a. [a]	n.a.
51	0 (0)*	n.a. [a]	n.a.
NOEC	12 mg a.s./kg	6.6 mg a.s./kg	6.6 mg a.s./kg
LC_{50}/EC_{50} (95% C.I.)	17 mg a.s./kg (16 – 19 mg a.s./kg)	> 12 mg a.s./kg (n.a.)	9.4 mg a.s./kg (6.8 – 12 mg a.s./kg)

SD…Standard deviation, n.a…not applicable
* Statistically significant compared to the control, based on Wilcoxon's Rank Sum Test with Bonferroni's Adjustment.
** Significantly reduced compared to the control, based on Bonferroni's t-Test.
*** Significantly reduced compared to the control, based on Bonferroni's t-Test. However, due to the lack of dose-response at the next highest treatment level, this reduction is not considered to be toxicant-related.
[a] Treatment level was excluded from statistical analyses due to the survival effect observed.

Conclusion: 28 d NOEC = 6.6 mg a.s./kg (growth, reproduction)
28 d LC_{50} = 17 mg a.s./kg (mortality)
28 d EC_{50} = 9.4 mg a.s./kg (reproduction)
28 d LOEC = 12 mg a.s./kg (growth, reproduction)
based on mean measured concentrations

Comment RMS: The observed significant reduction of average number of offspring per female amphipod at the test concentration of 3.0 mg a.s./kg was not considered for the NOEC determination because the effect did not establish a dose-response relationship. Hence, the NOEC was determined to be 6.6 mg a.s./kg,.

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

Endpoint	Classification Criteria according CLP (2nd ATP) (criteria in bold)		Evidence for fenpyrazamine
Degradation Fenpyrazamine	Hydrolotic degradataion of Fenpyrazamine pH 4: stable at 50°C pH 7: 32.5 d at 50 °C (a.s.) pH 9: 11 d at 25 °C Photodegradation of fenpyrazamine was fast with an experimental half-life of 1.6 days under the test conditions. Fenpyrazamine is not readily biodegradable, and does not meet the criterion for rapid degradation in a water/sediment study with a DT_{50} whole system of 35.5 days. Based on available data a non rapid degradation is proposed for fenpyrazamine.		The active substance is **not considered as ready biodegradable/rapid degradable** according to the CLP Regulation.
Bioaccumulation Fenpyrazamine	**Log K_{ow} is < 4** Fenpyrazamine Log K_{ow} =3.52at pH 7.2 and 25 °C		The measured log P_{OW} is 3.52 (at pH 7.2 and 25 °C) and is below the classification criteria of 4 (CLP) but above the classification criteria of 3 (DSD), therefore fenpyrazamine is considered to have **a moderate bioaccumulation potential.**
Acute aquatic toxicity Fenpyrazamine	**E_rC_{50} < 1 mg/L (algae)** **EC_{50} < 1 mg/L (marine aquatic invertebrates)**		Fenpyrazamine is of moderate toxicity to green algae (E_rC_{50} > 0.9 mg/L), but of high toxicity to diatoms (E_rC_{50} < 1 mg/L). In addition, the active substance is of low toxicity to fish (LC_{50} > 1 mg/L) but of high toxicity to marine invertebrates (EC_{50} < 1 mg/L) and fulfills the criteria for the proposed classification as **H400 (M = 10)** according to Regulation EC 1272/2008 are met.
Chronic aquatic toxicity Fenpyrazamine	**For not rapidly degradable substances: NOEC ≤ 0.1 mg/L**		Fenpyrazamine is of high chronic toxicity to fish and aquatic invertebrates (marine species) with a NOEC < 0.01 mg/L. In addition the active substance is of high chronic toxicity to algae (marine and freshwater diatoms) with a NOEC < 0.01 mg/L. Therefore fenpyrazamine fulfills the criteria for the proposed classification as **H410 (M = 10)** according to Regulation EC 1272/2008.
	Cyprinodon variegatus (marine fish)	NOEC = 0.062 (based on growth)	
	Americamysis bahia (marine mysid)	NOEC = 0.024 mg/L (based on growth)	

Endpoint	Classification Criteria according CLP (2nd ATP) (criteria in bold)		Evidence for fenpyrazamine
	Navicula pelliculosa (diatom)	NOEC = 0.0049 (based on yield) NOEC = 0.074 (based on growth rate)	
SUMMARY	**H400 (M = 10) / H410 (M = 10)**		**PROPOSED CLASSIFICATION**

Conclusion of environmental classification according to Regulation EC 1272/2008

Pictogram: GHS 09

Signal word: Warning!

Aquatic Acute 1, M = 10

Aquatic Chronic 1, M = 10

H400 'Very toxic to aquatic life'

H410 'Very toxic to aquatic life with long lasting effects'

Justification for the proposal

H400 follows from the toxicity of the active substance Fenpyrazamine to aquatic invertebrates (*Americamysis bahia*, LC_{50} = 0.83 mg/L, Fournier, 2010b), bivalves (*Crassostrea virginica*, EC_{50} = 0.66 mg/L, York, 2010) and algae (*Navicula pelliculosa*, E_rC_{50} = 0.202 mg/L and *Skeletonema costatum*, E_rC_{50} = 0.034 mg/L, Softcheck, 2010ab). Based on the high acute toxicity to the marine algae *Skeletonema costatum* (E_rC_{50} = 0.034 mg/L) a multiplication factor of 10 is proposed.

H410 follows from the toxicity of the active substance Fenpyrazamine to fish (*Cyprinodon variegatus*, NOEC = 0.062 mg/L based on growth, Lee, 2010a), aquatic invertebrates (*Americamysis bahia*, NOEC = 0.024 mg/L based on growth, Lee, M.R., 2010b) and algae (*Navicula pelliculosa*, NOEC = 0.0049 mg/L based on yield and 0.074 mg/L based on growth rate, Softcheck, 2010b). In addition, the active substance is not readily biodegradable (Burwood, C. & Scholey, A., 2006) and not rapidly biodegradable (Lewis, C.J. & Troth, K., 2007f). In the water-sediment study a DT_{50} of 35.5 days (geomean) was determined for the whole system. Also Fenpyrazamine does not meet the criterion of rapid degradation > 70 % within a 28-day period the aquatic environment. Based on the high chronic toxicity to the algae *Navicula pelliculosa*, (NOEC = 0.0049 mg/L) and the not rapidly degradation of the active substance in the water/sediment system a multiplication factor of 10 is proposed.

Based on the fish bioaccumulation study (Panthani, A.M., Herczog, K.J.S., 2007) with *L. macrochirus* a BCF (whole fish) of 9 was determined, which indicate a low potential to bioaccumulate in the aquatic food chain. The substance Fenpyrazamine does not meet the CLP criteria (BCF $\geq$ 500) based on the measured fish BCF.

Fenpyrazamine fulfils the criteria for classification as aquatic environmental hazard based on the CLP Regulation and should be classified.

The statements **P273**, **P391** and **P501** follow a general precautionary approach for dangerous substances.

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

Fenpyrazamine was hydrolytically stable at environmental temperature at pH 4 and 7 but at pH 9, degradation to S-2188-DC and subsequently to S-2188-OH occurred. At 20ºC and pH 9 the DT_{50} for hydrolysis was 24 days which is longer than the 16 days trigger for rapidly hydrolysed compounds. In contrast S-2188 was rapidly photolysed in aqueous solution, with experimental

DT_{50} values of 1.7 days. The main photolytic products were S-2188-DC (max. 63.8% after 7 days) and MCNI (max 17.7% after 30 days).

Fenpyrazamine is not readily biodegradable and cannot be classified as rapidly degraded in water sediment systems since less than 70 % is degraded within 28 days ($DT_{50whole\ system}$ of 35.5 days). Furthermore, mineralisation of the active substance is below 10 % of AR after 100 days after application.

Fenpyrazamine has a low potential of bioaccumulation in aquatic system because of a measured fish BCF of 9 (Panthani, A.M., Herczog, K.J.S., 2007).

Fenpyrazamine is acute and chronic toxic to aquatic organisms (aquatic invertebrates, algae).

Hazard pictogram		Environment
Hazard class and category:	Hazardous to the aquatic environment, Acute Hazard Category 1, Chronic Hazard Category 1	
Signal word	Warning!	
Hazard statement:	H400	Very toxic to aquatic life
	H410	Very toxic to aquatic life with long lasting effects
Precautionary statements - Prevention	P273	Avoid release to the environment
Precautionary statements - Response	P391	Collect spillage
Precautionary Statement Disposal	P501	Proper disposal of contents/container

6 OTHER INFORMATION

7 REFERENCES

7.1 Physico-chemical properties

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant), Published or not	Data Protection Claimed Y/N-R/NR	Owner
Asada, Y	2010	Explosive properties of S-2188 (Sumitomo QNP-0019) Not GLP, Unpublished	Y	SUM
Beckwith, R.C. & DiFrancesco, D.	2005	Determination of Dissociation Constant (pKa) - S-2188 Ricerca Biosciences LLC, Report No. 018410-1 (Sumitomo QNP-0001) GLP, Unpublished	Y	SUM
DiFrancesco, D.	2006	Determination of vapour pressure - S-2188 Ricerca Biosciences LLC, Report No. 018435-1 (Sumitomo QNP-0004) GLP, Unpublished	Y	SUM
Lentz, N.R.	2005a	Determination of water solubility - S-2188 Ricerca Biosciences LLC, Report No. 018315-1 (Sumitomo QNP-0003) GLP, Unpublished	Y	SUM
Lenz, N.R.	2005b	Determination of n-Octanol/Water Partition Coefficient - S-2188 Ricerca Biosciences LLC, Report No. 018434-1 (Sumitomo QNP-0002) GLP, Unpublished	Y	SUM
Lewis C.J.	2007	[14C]S-2188: Hydrolytic Stability Covance Laboratories Ltd, Report No. 0333/257-D2149 (Sumitomo QNM-0017) GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007d	[14C]S-2188: Photodegradation and Quantum Yield in Sterile, Aqueous Solution Covance Laboratories Ltd, Report No. 0333/258-D2149 (Sumitomo QNM-0029) GLP, Unpublished.	Y	SUM
Liney, P. & Jarvis, T.	2009	S-2188 – Stability in Air Exponent International Ltd., (Sumitomo QNM-0032) Not GLP, Unpublished	Y	SUM

Liney, P. & Jarvis, T.	2009	S-2188 – Oxidising Properties Assessment of Structure Exponent International Ltd., (Sumitomo QNP-0008) Not GLP, Unpublished	Y	SUM
Sweetapple, G.G. & Lentz, N.R.	2006a	Determination of Physical-Chemical Properties of S-2188PAI (amended report) Ricerca Biosciences LLC, Report no. 019388-1-1 (Sumitomo QNP-0006) GLP, Unpublished	Y	SUM
Sweetapple, G.G. & Lentz, N.R.	2006b	Determination of Physical-Chemical Properties of S-2188TGAI (amended report) Ricerca Biosciences LLC, Report no. 019387-1-1 (Sumitomo QNP-0007) GLP, Unpublished	Y	SUM
Weissenfeld, M	2009	S-2188 Technical Grade: Determination of the Relative Self-Ignition Temperature Harlan Laboratories Ltd, Report no. C40706 (Sumitomo QNP-0014) GLP, Unpublished	Y	SUM

7.2 Environmental hazard assessment

7.2.1 Fate and Behaviour in the environment

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**
Burwood, C. & Scholey, A.	2006	S-2188: Assessment of ready biodegradability by measurement of carbon dioxide evolution Covance Laboratories Ltd, Report No. 0333/261-D2149 Sumitomo Chemical Co., Ltd. QNM-0011 GLP, Unpublished.	Y	SUM
Jarvis, T. & Callow, B	2009a	Determination of normalised rates of degradation for S-2188 from four soils incubated under laboratory conditions Sumitomo Chemical Co. Ltd: QNM-0037 Non-GLP, Unpublished	Y	SUM
Jarvis, T. & Callow, B	2009b	Determination of rates of degradation for S-2188 from a water sediment study incubated under laboratory conditions. Sumitomo Chemical Co. Ltd: QNM-0040 Non-GLP, Unpublished	Y	SUM
Lewis, C.J.	2007	[^{14}C]S-2188: Hydrolytic Stability Covance Laboratories Ltd, Report No. 0333/257-D2149 Sumitomo Chemical Co., Ltd. QNM-0017 GLP, Unpublished.	Y	SUM

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
Lewis, C.J. & Scholey, A.	2006a	[^{14}C]S-2188: Aerobic soil metabolism and degradation Covance Laboratories Ltd, Report No. 0333/256-D2149 Sumitomo Chemical Co., Ltd. QNM-0016 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Scholey, A.	2006b	[^{14}C]S-2188: Adsorption/Desorption in soil Covance Laboratories Ltd, Report No. 0333/255-D2149 Sumitomo Chemical Co., Ltd. QNM-0012 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007c	[^{14}C]S-2188: Photodegradation on a soil surface Covance Laboratories Ltd, Report No. 0333/259-D2149 Sumitomo Chemical Co., Ltd. QNM-0020 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007e	[^{14}C]S-2188: Photodegradation and quantum yield in sterile, aqueous solution Covance Laboratories Ltd, Report No. 0333/258-D2149 Sumitomo Chemical Co., Ltd. QNM-0029 GLP, Unpublished.	Y	SUM
Lewis, C.J. & Troth, K.	2007f	[^{14}C]S-2188: Degradation and retention in water-sediment systems Covance Laboratories Ltd, Report No. D2149-0333/260 Sumitomo Chemical Co., Ltd. QNM-0028 GLP, Unpublished.	Y	SUM
Peatman, M.H.	2008	S-2188: Storage stability of residues in EU soil stored deep frozen Covance Laboratories Ltd, Report No. 0333/268-D2149 Sumitomo Chemical Co. Ltd Report No.: QNM-0036 GLP, Unpublished.	Y	SUM
Peatman, M.H. & Brice, A	2009	S-2188: The Dissipation of Residues in Soil in Northern and Southern Europe Covance Laboratories Ltd, Report No. 0333/266-D2149 Sumitomo Chemical Co. Ltd Report No.: QNM-0038 GLP, Unpublished.	Y	SUM

7.2.2 Aquatic Toxicity

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
Cafarella, M.A.	2006a	S-2188 Technical Grade – Acute Toxicity to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA)	Y	SUM

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
		Wareham, MA, USA Report No.: 13048.6504 (Sumitomo QNW-0002) GLP, Unpublished		
Cafarella, M.A.	2006b	S-2188 Technical Grade – Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6505 (Sumitomo QNW-0006) GLP, Unpublished	Y	SUM
Cafarella, M.A.	2006c	S-2188 Technical Grade – Early Life-Stage Toxicity Test with Rainbow Trout (*Oncorhynchus mykiss*) Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6506 (Sumitomo QNW-0011) GLP, Unpublished	Y	SUM
Fournier, A. E.	2010a	V-10135 T.G. (S-2188 T.G.) Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Static Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6301 (Sumitomo QNW-0048) GLP, Unpublished	Y	SUM
Fournier, A. E.	2010b	V-10135 T.G. (S-2188 T.G.) Acute Toxicity to Mysid (*Americamysis bahia*) Under Static Conditions, Following OPPTS Guideline 850.1035 Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6303 (Sumitomo QNW-0047) GLP, Unpublished	Y	SUM
Hoberg, J.R.	2006a	S-2188 Technical Grade – Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata* Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6509 (Sumitomo QNW-0004) GLP, Unpublished	Y	SUM
Lee, M.R.	2010a	V-10135 T.G. (S-2188 T.G.): Early Life-Stage Toxicity Test with Sheepshead Minnow (*Cyprinodon variegatus*), Following OPPTS Guideline 850.1400 Smithers Viscient Laboratories (formerly Springborn Smithers Laboratories (USA)) Report No.: 12709.6292 (Sumitomo QNW-0050) GLP, Unpublished	Y	SUM
Lee, M.R.	2010b	V-10135 T.G. (S-2188 T.G.): Life-Cycle Test with Mysids (*Americamysis bahia*) Following Draft OPPTS Guideline 850.1350 Smithers Viscient Laboratories (formerly Springborn Smithers Laboratories (USA)) Report No.: 12709.6293 (Sumitomo QNW-0049) GLP, Unpublished	Y	SUM

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
Panthani, A.M. & Herczog, K.J.S.	2007	Bioconcentration of [^{14}C]S-2188 by Bluegill Sunfish (*Lepomis macrochirus*) Ricerca Biosciences, LLC, Environmental Sciences Department Concord, OH, USA Report No.: 019492-1 (Sumitomo QNM-0018) GLP, Unpublished	Y	SUM
Picard, C. R.	2010a	42-Day Toxicity Test Exposing Freshwater Amphipods (*Hyalella azteca*) to V-10135 (S-2188) Applied to Sediment Under Static-Renewal Conditions Following EPA Test Methods. Smithers Viscient Laboratories (formerly Springborn Smithers Laboratories (USA)) Report No.: 12709.6290 (Sumitomo QNW-0052) GLP, Unpublished	Y	SUM
Picard, C. R.	2010b	V-10135 (S-2188) - 28-Day Toxicity Test Exposing Estuarine Amphipods (*Leptocheirus plumulosus*) to a Test Substance Applied to Sediment Following EPA Test Methods. Smithers Viscient Laboratories (formerly Springborn Smithers Laboratories (USA)) Report No.: 12709.6291 (Sumitomo QNW-0053) GLP, Unpublished	Y	SUM
Putt, A.E.	2006a	S-2188 Technical Grade – Acute Toxicity to Water Fleas, (*Daphnia magna*) Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6507 (Sumitomo QNW-0007) Study No.: GLP, Unpublished	Y	SUM
Putt, A.E.	2006b	S-2188 Technical Grade – Full Life-Cycle Toxicity Test with Water Fleas, *Daphnia magna* Under Flow-Through Conditions Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 13048.6508 (Sumitomo QNW-0012) GLP, Unpublished	Y	SUM
Softcheck, K. A.	2010a	V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*, Following OPPTS Draft Guideline 850.5400 Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6307 (Sumitomo QNW-0056) GLP, Unpublished	Y	SUM
Softcheck, K. A.	2010b	V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa*, Following OPPTS Draft Guideline 850.5400 Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6304 (Sumitomo QNW-0057) GLP, Unpublished	Y	SUM

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
Softcheck, K. A.	2010c	V-10135 T.G. (S-2188 T.G.) 96-Hour Toxicity Test with the Freshwater Blue-Green Alga, *Anabaena flow-aquae*, Following OPPTS Draft Guideline 850.5400 Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6306 (Sumitomo QNW-0058) GLP, Unpublished	Y	SUM
Softcheck, K. A.	2010d	7-Day Toxicity Test with Duckweed (Lemna gibba) Following OPPTS Draft Guideline 850.4400 Springborn Smithers Laboratories (USA) Wareham, MA, USA Report No.: 12079.6305 (Sumitomo QNW-0055) GLP, Unpublished	Y	SUM
York, D. O.	2010	V-10135 T.G. (S-2188 T.G.): Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions, Following OPPTS Guideline 850.1025 Smithers Viscient Laboratories (formerly Springborn Smithers Laboratories (USA)) Report No.: 12709.6302 (Sumitomo QNW-0046) GLP, Unpublished	Y	SUM

8 ANNEXES