Section A7 – Ecotoxicological Profile Including Environmental Fate and Behaviour

Section A7 **Annex Point IIA, VII**	**Ecotoxicological profile including environmental fate and behaviour**

Section 7.1 **Annex Point IIA, VII.7**	**Fate and behaviour in water**

Section 7.1.1 **Annex Point IIA, VII.7.6**	**Degradation, initial studies**

Section 7.1.1.1 **Annex Point IIA, VII.7.6.2**	**Abiotic**

Section 7.1.1.1.1 **Annex Point IIA, VI.7.6.2.1**	**Hydrolysis as a function of ph and identification of breakdown products**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.1.1.1.1/01** Corgier, M.C. and Plewa, A.P. [^{14}C] – MB46030, Hydrolysis at 25°C. 16 March 1992. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA N, 161-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	GHS 634A	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	> 98.6% w/w	
3.1.4	Further relevant properties	[U-^{14}c-phenyl) labelled	
3.2	**Reference substance**	MB 46030 : purity 99.3 XXXX : purity 96.5	

Section 7.1.1.1.1 **Annex Point IIA, VI.7.6.2.1**	**Hydrolysis as a function of ph and identification of breakdown products**	
3.2.1 Initial concentration of reference substance		
3.3 Test solution	See tables A7.1.1.1.1-1 and A7.1.1.1.1-2	
3.4 Testing procedure		
3.4.1 Test system	See table A7.1.1.1.1-3. Bottles are kept in a thermo regulated incubator in the dark	
3.4.2 Temperature	25° ±1°C	**x**
3.4.3 pH	5, 7 and 9	
3.4.4 Duration of the test	30 days	
3.4.5 Number of replicates	2 per pH per sampling time	
3.4.6 Sampling	Samples taken at 0, 5, 9 14, 20, 26 and 30 days	
3.4.7 Analytical methods	The radioactivity content of the organic and aqueous phases were determined by LSC. The organic extracts were then analysed by TLC (thin layer chromatography) with radioactive detection and by radio-HPLC. Structural confirmation was obtained by NMR	
3.5 Preliminary test	No	
	4. RESULTS	
4.1 Concentration and hydrolysis values	See Tables A7.1.1.1.1-4a and b.	
4.2 Hydrolysis rate constant (k_h)	See Table A7.1.1.1.1-4b	
4.3 Dissipation time	See Table A7.1.1.1.1-5	
4.4 Concentration – time data		
4.5 Specification of the transformation products	See Table A7.1.1.1.1-6	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	[^{14}C]-Fipronil, specific activity 730 MBq.mmol-1, radiochemical purity >98.6% was used in this study. The study was conducted in the dark, under sterile conditions, at pHs 5, 7 and 9 at an initial concentration of 0.89mg/l of buffer solution. Duplicate samples were removed after 0, 5, 9, 14, 20, 26 and 30 days after treatment. All samples were extracted twice with 50ml of dichloromethane. The radioactivity content of the organic and aqueous phases were determined by LSC. The organic extracts were then analysed by TLC (thin layer chromatography) with radioactive detection and by radio-HPLC. Structural confirmation was obtained by NMR.	

Section 7.1.1.1.1 **Annex Point IIA, VI.7.6.2.1**	**Hydrolysis as a function of ph and identification of breakdown products**

5.2	**Results and discussion**	The radioactivity of the treatment solution was found to be 4.016µCi corresponding to 89.05µg. Therefore the hydrolysis experiment was conducted at an initial concentration of 0.89 µg fipronil/ml of buffer solution. The radioactive recovery, for each individual sample, ranged from 96.4 to 101.6%; no radioactivity was found in the traps at T=30 days, therefore, no volatile compounds were formed at all pHs studied. The radioactivity recovered in the aqueous phases after extraction was always <1% of the initial radioactivity. At pHs 5 and 7, TLC and HPLC data showed that fipronil was hydrolytically stable (fipronil ≥ 98.5% of the applied radioactivity) At pH 9, fipronil was converted exclusively to XXXX, its XXXX analogue. The sum of both compounds represented more than 99% of the applied radioactivity, at all sampling times. 13F-NMR confirmed the identities of fipronil and XXXX, its XXXX analogue. The rate of conversion is best modelled by pseudo-first order kinetics with a half life of 28 days and a rate constant k = 0.0243 day^{-1}.	
5.2.1	k_H	pH 5: Stable pH 7 : nearly stable (2% loss in 30 days) pH 9 : k = 0.0243 day^{-1}	
5.2.2	DT_{50}	pH 5 and 7 : not applicable pH 9 : DT_{50}= 28 days	
5.2.3	r^2	- 0.9995	
5.3	**Conclusion**	Fipronil is hydrolytically stable at environmentally relevant pH values	**X**
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.1.1.1.1-1 Type and composition of buffer solutions (specify kind of water if necessary)

pH	Type of buffer (final molarity)	Composition
5	20 ~~nmoles/l~~ **mmoles/l**	8.4 g of citric acid dissolved in deionised water pH adjusted with 2N NaOH made up to 2000ml with water and the pH rechecked
7	20 ~~nmoles/l~~ **mmoles/l**	2.72 g of imidazole dissolved in deionised water pH adjusted with N HCl made up to 2000ml with water pH rechecked
9	20 ~~nmoles/l~~ **mmoles/l**	8.0 g of di-natrium tetraborate dissolved in deionised water pH adjusted with N HCl made up to x 2000ml with water pH rechecked

X

Table A7.1.1.1.1-2 Description of test solution

Criteria	Details
Purity of water	Purified, filtered deionised water with a resistivity ≥ 18MΩ.cm.
Preparation of test medium	1 ml of a solution containing MB 46030 at a nominal concentration of 90µg/ml added to 99ml of buffer solution
Test concentrations (mg a.i./L)	0.89mg/l
Temperature (°C)	25°C
Controls	None
Identity and concentration of co-solvent	1% acetonitrile
Replicates	2 per pH per sampling time

Table A7.1.1.1.1-3 Description of test system

Glassware	100 ml borosilicate glass bottles, fitted with screw caps and synthetic protective jackets absorbing UV radiation up to 360nm. 100 ml borosilicate glass bottles, fitted with two holed rubber bungs allowing two glass tubes to be fitted with two stopcocks
Other equipment	pH meter fitted with a combined glass electrode and a temperature probe and calibrated with standard buffers at pH's 4, 7 and 10. Steriliser, model AVX Laminar flux hood model FLV BAC Liquid Scintillation Counter Tricarb 4000 HPLC 1090 Hewlett-Packard, fitted with a UV Visible detector
Method of sterilisation	The buffers, the bottles and the requisite glassware and magnets were sterilized at a minimum temperature of 120°C for 20 minutes

Table A7.1.1.1.1-4a Hydrolysis of Test Compound, Transformation Products Expressed as Percentage of Initial Concentrations at pH5 and pH7 (mean values)

Compound	Sampling times			
	pH 5.0		pH 7.0	
	0	30	0	30
Parent compound	100.00	99.73	98.03	97.67
XXXX	-	0.00	-	1.08
Volatiles	-	0.00	-	0.00
total % recovery	100.00	99.73	98.03	98.75

Table A7.1.1.1.1-4b Hydrolysis of Test Compound, Transformation Products and Reference Substance, Expressed as Percentage of Initial Concentrations at pH9

Compound	Sampling times						
	0	5	9	14	20	26	30
Parent compound	99.07	87.33	79.30	70.25	61.60	52.93	47.54
XXXX	0.25	11.80	20.05	29.22	37.69	46.17	51.70
Volatiles	-	-	-	-	-	-	0.00
Total % recovery	99.32	99.13	99.35	99.47	99.29	99.10	99.24

Table A7.1.1.1.1-5 Dissipation times of parent compound, transformation products and reference compound at pH5, pH7 and pH9

	pH5		pH7		pH9	
	DT_{50}	DT_{90}	DT_{50}	DT_{90}	DT_{50}	DT_{90}
Parent compound	>30 days	>30 days	>30 days	>30 days	28 days	>30 days
XXXX	na	na	na	na	na	na

Table A7.1.1.1.1-6 Specification and Amount of Transformation Products

CAS-Number	CAS and/or IUPAC Chemical Name(s)	Amount [%] of parent compound measured at		
		pH5	pH7	pH9
None	XXXX	0.00	0.00	51.7

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and methods	Applicant's version is acceptable with the following comment: **3.4.2 Temperatures** A temperature range of 23.8 – 25.4 was recorded during the test, thus the temperature has not been maintained constant to at least ± 0.5 °C as recommended by the OECD guideline 111. Moreover, the test has been conducted at only one temperature whereas three temperatures in the range of 10-70°C are recommended by this guideline. However, these deviations are not considered as having affected the outcome of the study in a significant way and they are minor deviations.
Results and discussion	Agree with the applicant's version.
Conclusion	Applicant's version is acceptable with the following revision: **5.3 Conclusion** "Hydrolyse of fipronil is pH-dependant: at pH 5 and pH 7 *fipronil is hydrolytically stable* ~~*at environmentally relevant pH values*~~ and at pH 9 fipronil is unstable with only XXXX as breakdown product."
Reliability	1
Acceptability	Acceptable
Remarks	Errors in Table A7.1.1.1.1-1 were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.1.1.2 **Annex Point IIA, VI.7.6.2.2**	**Phototransformation in water including identity of the products of transformation**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.1.1.1.2/01** Corgier, M.M.C. and Plewa, A.P. ^{14}C –MB46030, Aqueous photolysis. 15 May 1992 (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	US EPA 161-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	GHS 634A	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	>97.5	
3.1.4	Radiolabelling	Specific activity 730 MBq.mmole^{-1}	
3.1.5	UV/VIS absorption spectra and absorbance value	Not given	
3.1.6	Further relevant properties	z	
3.2	**Reference substances**		
3.3	**Test solution**	See Table A7.1.1.1.2-1.	
3.4	**Testing procedure**		
3.4.1	Test system	See Table A7.1.1.1.2-2.	
3.4.2	Properties of light source	See Table A7.1.1.1.2-2.	
3.4.3	Determination of irradiance	Parker and Leahy method.	

Section 7.1.1.1.2 Annex Point IIA, VI.7.6.2.2	**Phototransformation in water including identity of the products of transformation**	
3.4.4 Temperature	25 ± 1°C	
3.4.5 pH	pH 5	
3.4.6 Duration of the test	6 hours	x
3.4.7 Number of replicates	Duplicates	
3.4.8 Sampling	At 0, 1, 2, 4 and 6 hours	
3.4.9 Analytical methods	The organic phases were quantitatively analysed by thin-layer chromatography (TLC) with radioactive detection and by high-pressure liquid chromatography (HPLC) with UV and radioactive detection. The aqueous phases were analysed by HPLC. ^{19}F-NMR.was used to confirm the nature and molar proportions of XXXX, XXXX and Rf = 0.37 product.	
3.5 Transformation products	Yes XXXX and XXXX	
	4. RESULTS	
4.1 Screening test	Not performed	
4.2 Actinometer data	Not applicable	
4.3 Controls	incubated for 6 hours in the dark	
4.4 Photolysis data		
4.4.1 Concentration values	0.9 mg/l	
4.4.2 Mass balance	99.8 – 103.0%	
4.4.3 k^c_p	-0.0176 days	x
4.4.4 Kinetic order	pseudo first order	
4.4.5 k^c_p / k^a_p	Not applicable	
4.4.6 Reaction quantum yield (ϕ^c_E)	not given in report	
4.4.7 k_{pE}	One lamp hour is equivalent to 0.091 hours of summer sunlight in Florida	x
4.4.8 Half-life ($t_{½E}$)	0.33 days in 'Summer sunlight in Florida	
4.5 Specification of the transformation products	See Table a7.1.1.1.2-3.	

Section 7.1.1.1.2 Annex Point IIA, VI.7.6.2.2	Phototransformation in water including identity of the products of transformation	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	[^{14}C]-Fipronil, specific activity 730 MBq.mmol^{-1}, radiochemical purity >97.5% was used in this study. The aqueous photolysis was carried out at pH5, at 25 ± 1°C, under sterile conditions, at an initial concentration of 0.90 mg/l in 1% acetonitrile co-solvent. The light of the Xenon lamp was filtered with special ultra violet glass to remove radiation below 290 nm. The time under the lamp was then converted to equivalents of "Florida summer days". Incubations under the lamp were conduced for 0, 1, 2, 4 and 6 hours. Control samples with test material were incubated in the dark for 6 hours. Each sample was extracted twice with dichloromethane and the radioactive content measured in the organic and aqueous phases and in the traps. The organic phases were quantitatively analysed by TLC (thin layer chromatography) with radioactive detection and by radio HPLC. The aqueous phases were analysed by HPLC. Confirmation of structural identity was obtained by ^{19}F-NMR.	
5.2 Results and discussion	The total radioactivity recovered for each individual sample ranged from 99.1 to 103.5% of the initial radioactivity. The maximum percentage of applied radioactivity content found in the traps was 0.02% in the ethylene glycol monomethyl ether and 0.04% in the NaOH traps. Thus practically no volatile compounds were formed during the photolysis experiments. Two degradation products were formed. After 6 hours of incubation, the major organic extract photo-product was XXXX (43.4 % of the applied radioactivity) and a minor component (HPLC RT = 2 min) accounting for 4.0% of applied radioactivity. The aqueous extract photo-products XXXX and a minor component (HPLC RT = 3.3 min) accounting for 8.2 and 5.6% of applied radioactivity, respectively. Parent material fipronil accounted for 32.3% of the radioactivity. Dark control incubations showed no appreciable degradation of fipronil after 30 days. Structural confirmation was obtained by NMR. The kinetics of photolytic degradation were first order with a half-life of 3.63 hours under the xenon lamp corresponding to 0.33 days of summer sunlight in Florida and a rate constant k = 0.0176 days-1. Photolysis can be considered a major route of fipronil degradation should it reach the aqueous environment. The quantum yield of direct photolysis of fipronil in aqueous solution was determined by a radiometer irradiation apparatus at 300nm. The quantum yield was 1.99 X 10-1 (mean of two values)	**X**
5.2.1 k^c_p	-0.0176 days	**X**
5.2.2 K_{pE}	One lamp hour is equivalent to 0.091 hours of summer sunlight in Florida	
5.2.3 ϕ^c_E	Not given in report	
5.2.4 $t_{½E}$	0.33 days in 'summer sunlight in Florida	

Section 7.1.1.1.2 **Annex Point IIA, VI.7.6.2.2**	**Phototransformation in water including identity of the products of transformation**	

5.3 Conclusion		**X**
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A7.1.1.1.2-1 Description of test solution and controls

Criteria	Details
Purity of water	Deionised water further purified and filtered via an Elgastat UHP apparatus. Its resistivity was ≥ 18 MΩ.cm
Preparation of test chemical solution	Dissolution of 0.9µg/ml plus 1% acetonitrile (co-solvent) in pH5 buffer
Test concentrations (mg a.s./L)	0.9 ~~µg/ml~~
Temperature (°C)	25°C
Preparation of a.s. solution	n.a
Controls	Sample kept for 30 days in the dark
Identity and concentration of co-solvent	1% acetonitrile

X

Table A7.1.1.1.2-2 Description of test system

Criteria	Details
Laboratory equipment	100 ml Pyrex photochemical reactors
Test apparatus	IL 1350 radiometer/photometer (International light)
Properties of artificial light source	
Nature of light source	Hanau (HERAEUS) Suntest fitted with a Xenon lamp
Emission wavelength spectrum	>290 nm
Light intensity	464 W/m^2
Filters	Ultra violet glass to remove radiation below 290 nm
Properties of natural sunlight	Not applicable
Latitude	-
Hours of daylight	-
Time of year	-
Light intensity	-
Solar irradiance (Lλ)	-

Table A7.1.1.1.2-3 Specification and Amount of Transformation Products

CAS-Number	CAS and/or IUPAC Chemical Name(s)	Amount [%] of parent compound measured at pH_5
None assigned	XXXX	43.4
None assigned	XXXX	~~3.2~~ **8.2**

X

X

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2006
Materials and methods	Agree with the applicant's version with the following comment: **3.4.6 Duration of the test** : According to OECD draft Guideline, the study duration should be sufficient to determine the DT_{75} of the test chemical and the formation and decline of major transformation products. Therefore the short test duration is considered as a deficiency.
Results and discussion	Agree with the applicant's version with the following revisions: **4.4.3 and 5.2.1** The rate constant is positive: k^{c}_{p}= 0.0176 $days^{-1}$ 4.4.7 kpE One lamp hour is equivalent to 0.091 ~~hours~~ **day** of summer sunlight in Florida **5.2 Results and discussion:** Information on monitoring of temperature and pH should be given, such as: "the temperature and pH range from 24.2 to 25.5 °C and 5.02 to 5.05 during the photodegradation, respectively." The last sentences of the section 5.2 ("*The quantum yield of direct photolysis of fipronil in aqueous solution was determined by a radiometer irradiation apparatus at 300nm. The quantum yield was 1.99 X 10-1 (mean of two values)*") are inconsistent with the study report because no indication of any calculations of quantum yield is given.
Conclusion	The applicant does not make any conclusions. The present study shows that sunlight can be considered as a major route of degradation of fipronil in aqueous environment. Under conditions of test, photolytic degradation of fipronil leads to formation of 4 different metabolites and among them only one, XXXX, is a major product.
Reliability	2 The reliability indicator has been reduced due to the short test duration.
Acceptability	Acceptable
Remarks	Unit of test concentration in Table A7.1.1.1.2-1 was deleted due to repetition. Errors in Table A7.1.1.1.2-3 was corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.1.2 **Annex Point IIA, VII.7.6.1**	**Biotic**

Section 7.1.1.2.1 **Annex Point IIA, VI.7.6.1.1**	**Ready biodegradability**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.1.1.2.1/01** Mead, C. Assessment of Ready Biodegradability: CO_2 Evolution Test 6 January 1997. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 301B EEC directive 92/69/EEC Annex V method C.4-C	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	9650027	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	95.2% w/w	
3.1.4	Further relevant properties	Water solubility of fipronil technical at 20°C = 1.9 @ pH 7	**X**
3.1.5	Composition of product	Not applicable	
3.1.6	TS inhibitory to micro-organisms	No Preliminary investigational work to assess any toxic effect of the test material on sewage sludge micro-organisms following the method described in the guideline could not be performed due to the insoluble nature of the test material. Therefore a toxicity control (fipronil and sodium benzoate) was included tin the study to assess any toxic effect of the material on the sewage sludge micro-organisms used in the study	x

Section 7.1.1.2.1 **Annex Point IIA, VI.7.6.1.1**	**Ready biodegradability**

3.1.7	Specific chemical analysis	CO_2 analysis was made using an TOC analyser. Each analysis was carried out in triplicate	
3.2	**Reference substance**	Sodium benzoate	
3.2.1	Initial concentration of reference substance	A standard material sodium benzoate was used. An initial stock solution of 1000 mg/litre was prepared by direct dispersion in culture medium and a 51.4 ml aliquot added to the test vessel to give a final test concentration of 17.1 mg/l, equivalent to 10 mg carbon/litre. A toxicity control (fipronil plus sodium benzoate) was included for validation purposes in addition to inoculum and standard sodium benzoate controls	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Table A7.1.1.2.1-2	
3.3.2	Test system	Table A7.1.1.2.1-3 The following test solutions were prepared and inoculated in 5 litre glass culture vessels each containing 3 litres of solution: a) a control, in duplicate, consisting of inoculated culture medium b) the standard material (sodium benzoate), in duplicate, in inoculated culture medium to give a final test concentration of 10mg carbon per litre. c) the test material, in duplicate, in inoculated culture medium to give a final test concentration of 19.05 mg carbon per litre d) the test material plus the standard material in inoculated culture medium to give a final concentration of 29.05 mg carbon per litre to act as a toxicity control (one vessel only).	
3.3.3	Test conditions	Table A7.1.1.2.1-4	
3.3.4	Method of preparation of test solution	The test material was prepared by direct dispersion in culture medium. An amount of test material (182.1 mg) was dispersed in inoculated culture medium and the volume adjusted to 3 litres to give a final concentration of 60.7 mg/litre equivalent to 19.05 mg carbon/litre.	
3.3.5	Initial TS concentration	60.7mg/l equivalent to 19.05 mg carbon/litre	
3.3.6	Duration of test	29 days	
3.3.7	Analytical parameter	CO_2 evolution	
3.3.8	Sampling	Samples (2ml) were taken from the first CO2 absorber vessel on days 0, 1, 2, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 27, 28 and 29. The second absorber vessel was sampled on days 0 and 29.	
3.3.9	Intermediates / degradation products	Not determined	
3.3.10	Nitrate/nitrite measurement	Not applicable to this method	
3.3.11	Controls	Inoculated culture medium no test substance	

Section 7.1.1.2.1 **Annex Point IIA, VI.7.6.1.1**	**Ready biodegradability**	

3.3.12 Statistics		
	4. RESULTS	
4.1 Degradation of test substance		
4.1.1 Graph	See Table 7.1.12.1-6	
4.1.2 Degradation	Fipronil 47% degradation after 28 days	
4.1.3 Other observations	No inhibition seen.	
4.1.4 Degradation of TS in abiotic control	See Table A7.1.1.1.2-6.	
4.1.5 Degradation of reference substance	See Table A7.1.1.1.2-6.	
4.1.6 Intermediates / degradation products	Not identified	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fipronil with a purity of 952 g/kg was used for this study. The test material was prepared by a direct dispersion in culture medium. An amount of test material (182.1) was dispersed in inoculated culture medium and the volume adjusted to 3 litres to give a final concentration of 60.7mg/l equivalent to 19.05 mg carbon/l. A standard material sodium benzoate was used. In initial stock solution of 1000 mg/l was prepared by direct dispersion in culture medium an a 51.4ml aliquot added to the test vessel to give a final test concentration of 17.1 mg/l, equivalent to 10 mg carbon/l. A toxicity control (fipronil and sodium benzoate) was included for validation purposes in addition to inoculum and standard sodium benzoate controls.	
	The following test solutions were prepared and inoculated in 5 litre glass culture vessels each containing 3 litres of solution: a) a control, in duplicate, consisting of inoculated culture medium b) the standard material (sodium benzoate), in duplicate, in inoculated culture medium to give a final test concentration of 10mg carbon per litre. c) the test material, in duplicate, in inoculated culture medium to give a final test concentration of 10.05 mg carbon per litre d) the test material plus the standard material in inoculated culture medium to give a final concentration of 29.05 mg carbon per litre to act as a toxicity control (one vessel only).	**x**

Section 7.1.1.2.1 **Annex Point IIA, VI.7.6.1.1**	**Ready biodegradability**

		Each test vessel was inoculated with the prepared inoculum at a final concentration of 30mg suspended solids. Test vessels were sealed and maintained in the dark at 21°C for 28 days. Degradation of the test material was assessed by quantification of carbon dioxide produced. Samples (2ml) were taken from the first CO2 absorber vessel on days 0, 1, 2, 3, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 27, 28 and 29. The second absorber vessel was sampled on days 0 and 29.	**X**
5.2	**Results and discussion**	Fipronil attained 47% degradation after 28 days. Sodium benzoate achieved total degradation after 28 day, confirming the suitability of the inoculum and test conditions. The toxicity control (fipronil plus sodium benzoate achieved 42% degradation, confirming the test substance was not toxic to the micro-organisms. According to OECD criteria a test material may be consider4ed to be readily degradable if > 60% degradation is attained after 28 days. Therefore, since there was only 47% degradation, fipronil cannot be considered readily degradable under the strict terms and conditions of the OECD guidelines	**x**
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	none	

Table A7.1.1.2.1-1 Guideline-Methods of EC and OECD for Tests on Ready/Inherent Biodegradability (According to OECD Criteria); Simulation Test

Test	EC-method	OECE-guideline	Test on ready/inherent biodegradability
CO_2 Evolution-Test (Modified Sturm Test)	**C.4-C**	**301B**	**ready**

Table A7.1.1.2.1-2 Inoculum / Test Organism

Criteria	Details
Nature	A mixed population of activated sewage sludge micro-organisms
Species	Not applicable
Strain	Not applicable
Source	Severn Trent Water Plc
Sampling site	Sewage treatment plant at Belpher, Derbyshire UK (which treats predominantly domestic sewage)
Laboratory culture	n.a

Method of cultivation	n.a
Preparation of inoculum for exposure	The sample of activated sewage sludge was maintained on continuous aeration upon receipt. A sample of the activated sewage sludge was washed 3 times by settlement and re-suspension
Pretreatment	Approximately 24 hours prior to addition of the test and standard materials the vessels were filled with 2400ml of culture medium and 30 ml of inoculum and aerated overnight
Initial cell concentration	60.7 mg/l equivalent to ~~10.05~~ **19.05** mg C/l

Table A7.1.1.2.1-3 Test System

Criteria	Details
Culturing apparatus	5 litre glass culture vessels containing 3 litres of solution
Number of culture flasks/concentration	2
Aeration device	CO_2- free air produced by sparking compressed air through the following series 3 x 500 ml dreschel bottles filled with 350 ml 10N NaOH 1 x 500 ml dreschel bottle filled with 350 ml 0.025N $Ba(OH)_2$ 1 x 500 ml empty dreschel bottle to prevent liquid carry -over
Measuring equipment	TOC analyser
Test performed in closed vessels due to significant volatility of TS	n.a

Table A7.1.1.2.1-4 Test Conditions

Criteria	Details
Composition of medium	As recommended in OECD Guidelines
Additional substrate	None
Test temperature	21°C
pH	7.4
Aeration of dilution water	70 ml/l
Suspended solids concentration	30 mg/l equivalent to 10 mg C/l
Other relevant criteria	n.a

Table A7.1.1.2.1-5 Pass Levels and Validity Criteria for Tests on Ready Biodegradability

	Fulfilled	Not fulfilled
Pass levels		

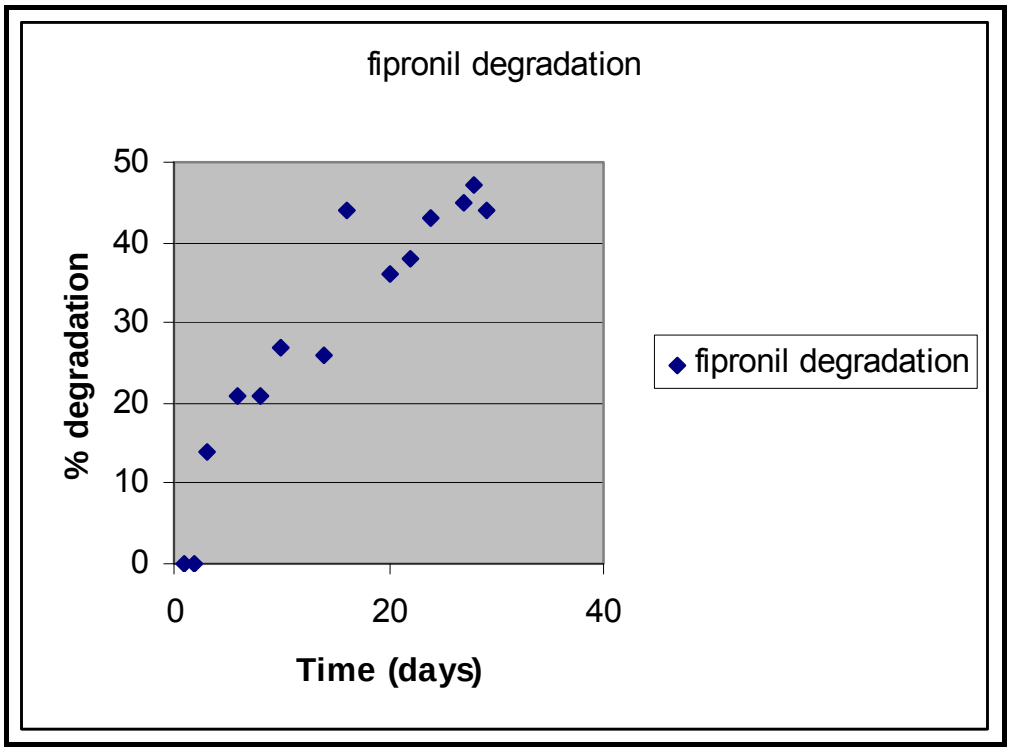
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$	✓	
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		✓
Criteria for validity		
Difference of extremes of replicate value of TS removal at plateau (at the end of test or end of 10-d window) < 20%	✓	
Percentage of removal of reference substance reaches pass level by day 14	✓	

Table A7.1.1.2.1-6 % Biodegradation Values

Day	% Degradation Sodium benzoate	% Degradation Fipronil	% Degradation Fipronil + Sodium benzoate Toxicity control
1	21	0	1
2	34	0	13
3	30	14	12
6	40	21	12
8	65	21	16
10	66	27	22
12	86	-	22
14	86	26	30
16	102**	44	31
20	91	36	27
22	98	38	33
24	101**	43	35
27	102**	45	40
28	105**	47	42
29*	100	44	49

- Value not determined
* Day 29 values corrected to include any carry-over of CO2 detected in absorber 2
** Degradation values in excess of 100% are considered to be due to sampling and/or analytical variation

Fig A7.1.1.2.1-1. Degradation curve for fipronil



EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with the applicant's version with the following correction: **3.1.4 Further relevant properties :** *Water solubility of fipronil technical at 20°C = 1.9* mg/L *@ pH 7.* **3.1.6 TS inhibitory to micro-organisms :** *…Therefore a toxicity control (fipronil and sodium benzoate) was included ~~tin~~* in *the study to assess any toxic effect of the material on the sewage sludge micro-organisms used in the study.*
Results and discussion	Agree with the applicant's version
Conclusion	Applicant's version is acceptable with the following revision: **5.1 Material and methods:** *c) the test material, in duplicate, in inoculated culture medium to give a final test concentration of ~~10.05~~* 19.05 *mg carbon per litre.* *d) … Each test vessel was inoculated with the prepared inoculum at a final concentration of 30mg/*L *suspended solids.* **5.2 Result and discussion:** *The toxicity control (fipronil plus sodium benzoate*) *achieved 42% degradation, confirming the test substance was not toxic to the micro-organisms* used in this study*. According to OECD criteria a test material may be ~~consider4ed~~* considered *to be readily degradable if > 60% degradation is attained after 28 days.* **5.3 Conclusion:** The applicant does not make any conclusions. The present study shows that fipronil cannot be considered readily degradable. It also shows that the test substance is not toxic to the micro-organisms used in this study.
Reliability	1
Acceptability	Acceptable
Remarks	Errors in Table A7.1.1.2.1-2 was corrected in bold and underlined
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.1.2.2 **Annex Point IIA, VII.7.6.1.2**	**Inherent biodegradability**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Inherent biodegradability is addressed in Section 7.1.2.2 (water sediment study)	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.1.2.3 **Annex Point IIIA, XII.2.1**	**Biodegradation in seawater**	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	None of the proposed uses would bring the compound into significant contact with sea water	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2 **Annex Point IIIA, XII.2.1**	**Rate and route of degradation in aquatic systems**

Section 7.1.2.1 **Annex Point IIIA, XII.2.1**	**Biological sewage treatment**

Section 7.1.2.1.1 **Annex Point IIIA7.1.2.1.1**	**Aerobic biodegradation**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Aerobic biodegradation in biological sewage treatment is addressed under Section 7.1.1.2.1 – Ready biodegradability and 7.4.1.4 – Toxicity to the activated sludge in a respiration test.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2.1.2 **Annex Point IIIA, XII.2.1**	**Anaerobic biodegradation**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	It is very unlikely that anaerobic aquatic system would be exposed to Fipronil when used according to the label recommendations (See Chapter 5). In addition data presented under AIII 7.4.1.4 showed that the activity of activated sludge was not affected at the highest concentration tested. Therefore, no anaerobic biodegradation study was performed.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2.2 **Annex Point IIIA, XII.2.1**	**Biodegradation in freshwater**

Section 7.1.2.2.1 **Annex Point IIIA, XII.2.1**	**Aerobic aquatic degradation study**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The behaviour of Fipronil in water compartment is adequately described by existing photolysis, hydrolysis and water/sediment studies. Therefore no further aerobic aquatic degradation study is required.	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2.2.2 Water/sediment degradation study **Annex Point IIIA, XII.2.1**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.1.2.2.2/01** Roohi, A. and Buntain, I. [^{14}C]-Fipronil: Degradation in Two Water/Sediment Systems 25 February 2002. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes SETAC Guidelines	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Fipronil (purified)	
3.1.1	Lot/Batch number	PJS1071/1	
3.1.2	Specification	Not given	
3.1.3	Purity	99.5% radiochemical purity	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[^{14}C]-Fipronil with a specific activity of 1213.6 MBq.mmol^{-1} with a radiochemical purity of 99.5% by TLC and 100% by HPLC was used in this study. Samples of sediment and water were collected fresh from a river at Iron Hatch, East Stock, Staffordshire, UK and from Ongar, Essex, UK and were stored at 4°C for ca. two weeks prior to use. The physico-chemical properties of the water and sediment are given in Table 7.1.2.2.2-1 and 2.	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

	The systems were incubated in glass flasks containing sediment and water at a ratio of 1:4.7 (Iron Hatch and 1:8.2 (Ongar), and were maintained in the dark at 20± ^{o}C with an aerobic water layer and anaerobic sediment. There was an acclimatisation period of four weeks prior to the addition of fipronil. [^{14}C]-Fipronil was applied to the water in 250µl acetonitrile containing 60 µg fipronil which was equivalent to 212 g/ha. Volatile products evolved from the surface were collected in a series of trapping solutions. The oxygen content of the water was maintained above 20% saturation and monitored regularly. The Iron Hatch sediment had an average redox potential of -238 mV, the water phase +228 mV and the pH was 7.8. The Ongar sediment had an average redox potential of -255mV, the water phase -216 mV and the pH was 7.4. Duplicate samples were analysed at a range of time periods up to a total of 163 days for the Ongar system and 244 days for the Iron Hatch system. The water was decanted and the sediment extracted with methanol or acetonitrile and then acetonitrile/water. Following concentration extracts were analysed by radio-HPLC and LC/MS.	X
5.2 Results and discussion	Good radiochemical recoveries were obtained from both systems with overall mean recoveries of 93.3% (Iron Hatch, range 90 – 97%) and 94.1% (Ongar, range 91 – 97%). Tables A7.1.2.2.2-3 and 4. In both systems the applied radioactivity partitioned steadily from the water into the sediment. Volatile products accounted for <1.5% of applied radioactivity in the Iron Hatch and 0.2% in the Ongar system. The quantity of unextracted radioactivity remained low throughout the experiment and was <5%. Water Phase In the water phase the only major metabolite was XXXX, the hydrolysis product. Small quantities of observed XXXX, XXXXand XXXXwere also observed. (See Tables A7.1.2.2.2-5 and 6). Confirmation of structural identities was obtained by LC/MS of all of the metabolites assigned structures. XXXXreached a maximum of 18.6% in the Iron Hatch system at 244 days. In the Ongar system it reached a maximum of 5.1% at 112 days before declining to 2.0% at 163 days. XXXXreached 5.3% in the Iron Hatch system at 112 days (falling to 6.4% at 244 days) and 7.4% in the Ongar system at 163 days. XXXX reached 2.2% in the Iron Hatch system at 244 days and 1.9% in the Ongar system at 28 days. In addition small amounts of XXXXand minor components were also detected (<5% of applied radioactivity) at various isolated time points in the water for both systems.)	X

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

Sediment Phase
In the sediment the only major metabolite was XXXX. Lesser quantities of XXXX, XXXXand XXXXwere also confirmed. XXXX reached a maximum of 21.0% in the Iron Hatch system at 163 days before falling to 16.9% at 244 days. In the Ongar system it reached a maximum of 54.7 at 163 days.

XXXX reached maxima of 1.7% in the Iron Hatch system at 163 days and 6.5% in the Ongar system at 163 days. XXXX reached 5.0% in the Iron Hatch system at 244 days and 2.3% in the Ongar system at 112 days.

In addition, small amounts of XXXX and minor components were also detected (<5% of applied radioactivity) at various isolated time points in the sediment for both systems. (See Tables A7.1.2.2.2-7 and 8).

Total System
In the total water/sediment three major metabolites were identified (See Tables A7.1.2.2.2-9 and 10). XXXX, XXXX (Iron Hatch only) and XXXX (Ongar only.)

XXXX reached a maximum of 29.3% in the Iron Hatch system at 163 days before falling to 23.2% at 244 days. In the Ongar it reached a maximum of 66.4% at 163 days.

XXXX reached a maximum of 7.0% in the Iron Hatch system at 163 days before falling to 3.7% at 244 days and a maximum of 14.2% in the Ongar system at 163 days. X

The proposed metabolic pathway is given in Figure A7.1.2.2.2-1. Fipronil is degraded in the water phase by hydrolysis to XXXXand in the sediment phase by reduction to XXXX. Small quantities (<4%) of the oxidation product XXXXwere observed.

Hydrolysis of the cyano moiety to the amide is a detoxification process.

The DT and DT values of fipronil using KIM model which provided a superior fit than first order kinetics.

Fipronil	**DT50 (days)**	**DT90 (days)**
Iron Hatch		
Water	32.8	319.3
Total system	76.0	347.3
Ongar		
Water	22.7	99.4
Total system	39.1	169.1

Section 7.1.2.2.2 Water/sediment degradation study **Annex Point IIIA, XII.2.1**		
	In an aerobic aquatic environment, fipronil partitions steadily into the underlying sediment where it degrades by reduction to XXXX. XXXX is further degraded by hydrolysis to XXXX. Fipronil is also hydrolysed to XXXX and, to a much lesser extent oxidised too XXXX. There is evidence than XXXX and XXXX are further transformed to XXXX via oxidation or hydrolysis respectively. The results of this study show that fipronil will not persist in an aerobic aquatic environment.	x
5.3 Conclusion		
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A7.1.2.2.2-1 Characterisation of Surface Waters

	Iron Hatch	**Ongar**
Soil reference	01/04	01/05
Total Nitrogen (ppm)	<0.05	<0.05
Total Phosphorous (ppm)	0.3	1.9
Total Organic Carbon (ppm)	28.5	23.8
Water hardness (ppm as $CaCO_3$)	184	106.0
Temperature ^oC	8.6	3.9
pH	7.3	8.1
Redox potential (mV)	281,286	224
Oxygen content below surface (%)	69	62

Table A7.1.2.2.2-2 Physico-chemical Properties of the Sediments

Soil reference		**01/04**	**01/05**
Source		Iron Hatch, Runoff Channel, East Stoke Staffordshire, UK	Boarded Barns Farm, Ongar, Essex, UK
Particle size Distribution (µm)		%	%
500 – 2000		37.55	5.79
250 – 500		53.88	15.79
106 – 250		5.25	13.78
63 – 106		0.41	5.38
20 – 63		0.68	11.49
2 – 20		0.77	15.87
<2		1.46	31.89
Total		**100.00**	**99.99**
Textural classification			
ADAS		Sand	Clay Loam
Sand	(60 – 2000 µm)	97.14	41.54
Silt	(2 – 60 µm)	1.40	26.56
Clay	(<2 µm)	1.46	31.89
USDA		Sand	Clay Loam
Sand	(50 – 2000 µm)	97.3	44.21
Silt	(2 – 50 µm)	1.24	23.89
Clay	(<2 µm)	1.46	31.89
Organic Carbon (%)		0.40	3.20
Organic Matter (%)		0.69	5.52
Cation Exchange Capacity		12.50	51.70
pH	Water	8.0	8.2
	KCl (1M)	8.0	8.0
	$CaCl_2$ (0.01M)	7.2	7.6
Total Nitrogen (mg/kg)		252	2591
Total Phosphorous (mg/kg)		193	1019
Microbial biomass (g C/g soil)			
	initial	50	136
	final	22	102

Table A7.1.2.2.2-3 Distribution and Recovery of Applied Radioactivity from Iron Hatch Water/Sediment System (mean values)

Mean Value	% of Applied Radioactivity				
	Water	Sediment		Volatiles	% Total
		Extracted	Unextracted		
0 hour	92.77				92.77
3 hours	93.22	1.41	0.12	0.02	94.78
18 hours	88.23	1.62	0.09	0.03	89.97
1 day	85.08	6.11	0.32	0.02	91.52
2 days	86.86	10.22	0.60	0.02	97.70
7 days	80.21	12.12	0.62	0.03	92.98
10 days	72.19	19.15	1.41	0.04	92.78
15 days	67.48	22.92	1.41	0.04	91.85
16 days	68.81	24.78	2.16	0.03	95.78
17 days	62.39	29.47	1.47	0.04	93.38
21 days	64.28	27.90	1.78	0.04	94.00
23 days	60.90	28.78	2.82	0.05	92.55
28 days	64.76	27.38	2.58	0.08	94.81
56 days	63.62	27.57	3.62	0.12	94.94
112 days	57.00	31.00	2.29	0.22	90.52
~~183~~ **163** days	49.84	39.08	3.79	0.25	92.96
244 days	50.92	37.15	4.03	1.19	93.29
Overall Mean					93.33

X

Table A7.1.2.2.2-4 Distribution and Recovery of Applied Radioactivity from Ongar Water/Sediment System (mean values)

Mean Value	% of Applied Radioactivity				
	Water	Sediment		Volatiles	% Total
		Extracted	Unextracted		
0 hour	95.65				95.65
3 hours	93.31	1.19	0.11	0.02	94.62
18 hours	83.47	6.97	0.67	0.02	91.14
1 day	90.09	3.59	0.31	0.02	94.00
2 days	77.70	16.51	1.28	0.02	95.51
7 days	69.27	22.16	2.11	0.04	93.57
10 days	51.42	41.92	2.77	0.04	96.15
15 days	63.44	26.02	2.77	0.03	92.26
16 days	48.08	45.15	4.45	0.05	97.72
17 days	72.40	19.87	1.50	0.04	93.82
21 days	63.08	25.77	2.11	0.05	91.00
23 days	58.23	31.31	2.91	0.06	92.51
28 days	63.74	28.02	2.61	0.06	94.42
56 days	35.62	52.07	6.79	0.13	94.61
112 days	23.86	~~86.47~~ **66.47**	4.27	0.18	94.78
163 days	17.84	71.64	4.32	0.19	93.99
Overall Mean					94.11

X

Table A7.1.2.2.2-5 Composition of Radioactivity in the Water Phase from the Iron Hatch System (mean values)

	% of Applied Radioactivity for each metabolite								
	Total in Water	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour	92.77	92.77							92.77
3 hours	93.22	93.22							93.22
18 hours	88.23	88.23							88.23
1 day	85.08	85.08							85.08
2 days	86.86	86.86							86.86
7 days	80.21	73.30		4.35	2.57				80.21
10 days	72.19	69.36		2.62		0.21			72.19
15 days	67.48	~~61.67~~ **61.87**		4.02	1.59				67.48
16 days	68.81	62.95		4.77	1.08				68.81
17 days	62.39	54.11	0.62	5.41	2.23				62.39
21 days	64.28	51.61		6.24	5.15		1	1.28	64.28
23 days	60.90	51.08		5.53	4.29				60.90
28 days	64.76	49.92		8.03	6.82				64.76
56 days	63.62	45.81		11.10	5.33		1	1.39	63.63
112 days	57.00	31.37	2.94	12.54	8.75	1.04	1	0.33	56.98
163 days	49.84	18.01	5.26	15.65	8.26	0.71	2	1.95	49.84
244 days	50.92	15.65	2.81	20.00	6.86	2.31	3	3.32	50.92

X

Table A7.1.2.2.2-6 Composition of Radioactivity in the Water Phase from the Ongar (mean values)

	% of Applied Radioactivity for each metabolite								
	Total in Water	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour	95.65	95.65							95.65
3 hours	93.31	93.31							93.31
18 hours	83.47	83.47							83.47
1 day	90.09	89.63		0.18		0.28			90.09
2 days	77.70	77.70							77.70
7 days	69.27	68.01			1.26				69.27
10 days	51.42	50.04		1.38					51.42
15 days	63.44	57.50	2.21		3.73				63.44
16 days	48.08	44.47	2.59		1.02				48.08
17 days	72.40	67.08	2.93		~~2.83~~ **2.38**				72.40
21 days	63.08	55.82	2.69		4.56				63.08
23 days	58.23	50.33	2.74		5.16				58.23
28 days	63.74	54.15	0.63	1.64	5.42	1.91			63.74
56 days	35.62	27.15	1.19	4.43	2.85				35.62
112 days	23.86	10.12	2.97	5.05	4.95	0.42	1	0.34	23.86
163 days	17.84	1.32	6.33	2.00	7.44	0.20	2	0.55	17.84

X

Table A7.1.2.2.2-7 Composition of Radioactivity in the Sediment Phase from the Iron Hatch System (mean values)

	% of Applied Radioactivity for each metabolite								
	Extracted	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour									
3 hours	1.41								1.41
18 hours	1.62								1.62
1 day	6.11	5.92			0.19				6.10
2 days	10.22	9.81			0.37	**0.05**			10.22
7 days	12.12	10.52			0.34	**1.27**			12.12
10 days	19.15	16.66	0.05	0.39	0.68	**1.37**			19.15
15 days	22.92	13.94	0.31	0.50	0.50	**7.67**			22.92
16 days	24.78	15.33	0.39	0.73	0.68	**7.66**			24.78
17 days	29.47	18.11	0.38	0.90	0.53	**9.55**			29.47
21 days	27.90	9.71	0.48	~~0.82~~ **0.62**	0.52	**16.58**			27.90
23 days	28.78	10.51	0.64	0.89	0.44	**16.29**			28.78
28 days	27.38	7.33	0.78	0.93	0.89	**17.44**			27.38
56 days	27.57	12.25	0.84	1.48	0.84	**12.16**			27.57
112 days	31.00	11.02	0.90	2.03	1.61	**15.45**			31.00
163 days	39.08	10.02	1.69	3.72	2.48	**21.03**	1	0.15	39.08
244 days	37.15	8.60	1.14	5.26	4.95	**16.85**	3	0.35	37.15

X

Table A7.1.2.2.2-8 Composition of Radioactivity in the Sediment Phase from the Ongar system (mean values)

	% of Applied Radioactivity for each metabolite								
	Extracted	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour									
3 hours	1.19								1.19
18 hours	6.97	6.97							6.97
1 day	3.59	3.59							3.59
2 days	16.51	16.27				0.24			~~18.51~~ **16.51**
7 days	22.16	11.39	0.16	0.22	10.01	0.39			22.16
10 days	41.92	31.13	0.56	1.19	8.19	0.85			41.92
15 days	26.02	4.89	0.35		20.55	0.23			26.02
16 days	45.15	26.49	0.87	1.25	15.20	1.35			45.14
17 days	19.87	4.86	0.04		14.69	0.28			19.87
21 days	25.77	5.52	0.52	0.11	19.45	0.17			25.77
23 days	31.31	4.24	0.31		26.76				31.31
28 days	28.02	5.78	0.64	0.18	21.43				28.02
56 days	52.07	16.89	1.51	1.25	31.20	1.23			52.07
112 days	66.47	11.30	3.78	2.44	~~48.39~~ **46.39**	2.23	2	0.34	66.47
163 days	64.78		6.48		54.69	1.61	1	2.00	64.78

X

Table A7.1.2.2.2-9 Composition of Radioactivity in the Total from the Iron Hatch system (mean values)

	% of Applied Radioactivity for each metabolite								
	Extracted	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour	92.77	92.77							92.77
3 hours	94.63	93.22							93.22
18 hours	89.86	88.23							88.23
1 day	91.18	90.99				0.19			91.18
2 days	97.08	96.66			0.05	0.37			97.08
7 days	92.33	83.81		4.35	3.84	0.34			92.33
10 days	91.34	86.02	0.05	3.01	1.37	0.90			91.35
15 days	90.40	75.82	0.31	4.53	9.26	0.50			90.41
16 days	93.59	78.28	0.39	5.50	8.74	0.68			93.59
17 days	91.86	72.23	1.00	6.31	11.78	0.53			91.86
21 days	92.19	61.32	0.48	6.86	21.73	0.52	1	1.28	92.19
23 days	89.68	61.59	0.64	6.42	20.58	0.44			89.68
28 days	92.14	57.25	0.78	8.96	24.26	0.89			92.14
56 days	91.20	58.06	0.84	12.58	17.49	0.84	1	1.39	91.20
112 days	88.00	42.39	3.84	14.57	24.19	2.65	1	0.33	87.97
163 days	88.92	28.02	6.95	19.37	29.29	3.19	2	2.09	88.92
244 days	88.07	24.25	3.94	25.25	23.71	7.26	3	3.67	88.08

X

Table A7.1.2.2.2-10 Composition of Radioactivity in the Total from the Ongar system (mean values)

	% of Applied Radioactivity for each metabolite								
	Extracted	**Fipronil**	XXXX	XXXX	XXXX	XXXX	**Number of components <5%**	**% of components <5%**	**Total**
0 hour	95.65	95.65							95.65
3 hours	94.50	93.31							93.31
18 hours	90.44	90.44							90.44
1 day	93.68	93.22		0.18		0.28			93.68
2 days	94.21	93.97				0.24			94.21
7 days	91.43	79.40	0.16	0.22	11.26	0.39			91.43
10 days	93.34	81.17	0.56	2.57	8.19	0.85			93.35
15 days	89.46	62.39	2.56		24.28	0.23			89.46
16 days	93.22	70.95	3.46	1.25	16.22	1.35			93.22
17 days	92.27	71.94	2.97		17.07	0.28			92.27
21 days	88.85	61.35	3.22	0.11	24.01	0.17			88.85
23 days	89.54	54.57	3.05		31.92				89.54
28 days	91.76	59.92	1.27	1.81	26.85	1.91			91.76
56 days	87.69	44.03	2.69	5.68	34.05				87.69
112 days	90.33	21.16	6.75	7.48	51.34	2.65	3	0.94	90.33
163 days	88.47	1.26	14.22	1.48	66.41	2.00	3	3.10	88.47

X

Figure A7.1.2.2.2-1 Proposed metabolic Pathway in Water/Sediment System

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with the applicant's version with the following comment: According to OECD Guideline, the water/sediment ratio should range from 3:1 to 4:1. Therefore the ratio of the Ongar system (1:8) is considered as a deficiency. Moreover, the OCDE Guideline indicates a depth of sediment of 2.5+/- 0.5 cm; the approximates depths of sediments giving in this study are 2 cm for the Iron Hatch system and 4 cm for the Ongar system, which can also be considered as a deficiency.
Conclusion	Applicant's version is acceptable with the following revision: **5.1 Material and methods** *The systems were incubated in glass flasks containing sediment and water at a ratio of ~~1:4.7~~ 1:4.1 (Iron Hatch) and 1:8.2 (Ongar), and were maintained in the dark at ~~20± °C~~ 20± 2°C with an aerobic water layer and anaerobic sediment.* **5.2 Result and discussion** *Water phase* XXXX *reached a maximum of ~~18.6%~~ 20% in the Iron Hatch system at 244 days. In the Ongar system it reached a maximum of 5.1% at 112 days before declining to 2.0% at 163 days.* XXXX *reached 5.3% in the Iron Hatch system at ~~112~~ 163 days (falling to ~~6.4%~~ 2.81% at 244 days) and ~~7.4%~~ 6,3% in the Ongar system at 163 days.* XXXX *reached ~~2.2~~ 2.3 % in the Iron Hatch system at 244 days and 1.9% in the Ongar system at 28 days.* *Total system* XXXX *reached a maximum of 7.0% in the Iron Hatch system at 163 days before falling to 3.7% at 244 days and a maximum of 14.2% in the Ongar system at 163 days.* XXXX reached a maximum of 25.25% in the Iron Hatch system at 244 days, and a maximum of 7.48% in the Ongar system at 112 days before falling to 1.48 % at 163 days. *Metabolic pathway: .. There is evidence than* XXXX *and* XXXX *are further transformed to* XXXX *via oxidation or hydrolysis respectively.*
Reliability	2 The reliability indicator has been reduced due to the water/sediment ratio of the Ongar system.
Acceptability	Acceptable
Remarks	Errors in Table A7.1.2.2.2-3, A7.1.2.2.2-4, A7.1.2.2.2-5, Table A7.1.2.2.2-6, Table A7.1.2.2.2-7, Table A7.1.2.2.2-8, Table A7.1.2.2.2-9, Table A7.1.2.2.2-10 were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	

Conclusion
Reliability
Acceptability
Remarks

Section 7.1.2.2.2 Water/sediment degradation study **Annex Point IIIA, XII.2.1**		

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.1.2.2.2/02** Feung C.S., Yenne S.P. Fipronil: Aerobic aquatic metabolism XXXX 27 March 1997.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA 162-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Fipronil	
3.1.1	Lot/Batch number	GHS-826	
3.1.2	Specification	Not given	
3.1.3	Purity	Radiochemical purity >97%	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[^{14}C]-Fipronil, Specific activity : 19.85 mCi.mmol^{-1} (10^5 dpm/µg), radiochemical purity >97% was used in this study. Chemical name (IUPAC): (+)-5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethanesulfinyl-1H-pyrazole-3-carbonitrile. The metabolism of [^{14}C]-fipronil under aerobic aquatic conditions in sediment and pond water was studied. The soil and water characteristics are given in Table 7.1.2.2.2-11	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	
	A mixture of a fresh sandy loam sediment and pond water (pH 7.3) obtained from Clayton Farm, North Carolina, USA, was treated with radiolabelled Fipronil (dissolved in methanol) to obtain a concentration of approximately 0.05 ppm in the water, corresponding to a use rate of 0.05 lb ai/acre (56 g ai/ha). Each test system contained about 25 g of sediment (based on dry weight) and approximately 50 mL of pond water. The treated hydrosoils were wrapped with aluminium foil and were aerobically incubated in the dark at 25 ± 1°C for one year to establish the decline of test substance and formation of degradates. The volatile trapping system consisted of an empty backflow trap, two organic-solvent (methanol or acetone) traps for trapping organic volatiles, another empty backflow trap, two 2-ethoxyethanol ethanolamine (2:1 v/v) traps for trapping carbon dioxide and an overflow trap. Radioactivity in the trapping systems was determined at each sampling interval. Duplicate sediment and water samples were analysed at intervals of 0, 7, 14 days and 30, 60, 90, 120, 180, 270, and 365 days after [^{14}C]-Fipronil application. In addition, two untreated samples were also sampled at 0 day and two at the last day of the experiment. The water phase of the duplicate samples was decanted and filtered and then, assayed for total radioactivity and extracted with organic solvents. Extraction of sediment using both methanol and acidic acetone was immediately performed at each sampling. The incubated sediment was extracted three times with approximately 50 mL of methanol. Then, the sediment was re-extracted three times with acetone:water:phosphoric acid (acidic acetone), (90:8:2, v/v/v). Analysis of methanol extract and acidic acetone extract was for radioactivity followed by HPLC chromatographic analysis. The soil residue was allowed to air-dry prior to combustion for ^{14}C-analysis.	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

5.2	**Results and discussion**	The data in 7.1.2.2.2-12 show that the average total recovery of [^{14}C]-residues in the sediment, pond water and volatiles ranged from ca. 95.70% to ca. 106.40% of the applied dose throughout the study. Average radioactivity in the pond water rapidly transferred/adsorbed to sediment, decreasing from an initial level of ca. 21% at day 0 to 4% at day 7. From day 14 onward, levels in water ranged from 1.6 to 3.8% of applied dose. The majority of radioactivity associated with the sediment (>62%) was extracted with methanol (ca. 74 - 88%) and lesser amounts in acidic acetone (4.2-15.0%). The average (unextracted) residue slowly increased from 0.06% on day 0 to a maximum of 7.41% on day 270, falling to 2.15% by the end of the study. Approximately 3% of applied radioactivity was collected in the traps designed to collect $^{14}CO_2$ and organic volatiles. The extractable [^{14}C]-Fipronil rapidly decreased from the initial applied dose 99.36% to ca. 4.07% at 60 days, with an estimated half-life of 14.5 days while the reduced sulfide compound XXXX increased to reach a plateau of about 81 - 85% after 3 months. Also identified as a minor metabolite was amide XXXX. Low levels of photometabolite XXXX and its amide XXXX were also tentatively identified, presumably artefacts from work-up. The acid XXXX (from further hydrolysis of XXXX) and amide XXXX (but designated XXXX in this report) (from hydrolysis of XXXX) were also tentatively proposed as minor degradates. The identity of Fipronil, XXXX, XXXX, XXXX and XXXX were confirmed by LC/MS.	
5.3	**Conclusion**	The results of this study showed that the majority of the test substance, [^{14}C]-Fipronil, was rapidly transferred/adsorbed to the sediment within 7 days of incubation with less than 4% in the water phase after 7 days. The half-life of [^{14}C]-Fipronil under aerobic aquatic conditions was 14.5 days. XXXX was found as the major metabolite in the sediment and accounted for <1% in the water phase.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.1.2.2.2-11 Soil and water characteristics

Property	Soil	Water
Soil classification USDA	Sandy Loam	
pH	5.80	7.3
CEC (meg/100g)	9.47	
% organic matter	8.00	
% sand	53.2	
% silt	31.6	
% clay	15.2	
Alkalinity (mg $CaCO_3$)		23
Total suspended Solids (mg.mL^{-1})		32
Hardness (mg $CaCO_3.L^{-1}$)		22
Dissolved Oxygen (mg D.O..L^{-1})		8.2
Ca (mg.L^{-1})		4
Mg (mg.L^{-1})		3
Microbial activity	260000 colonies/g	750000 colonies/mL
Redox potential (mV)	-149 to -157	162 to 295

Table A7.1.2.2.2-12 The Distribution of Radioactivity (% of applied)

Time (days)	Sample Number	Volatile traps		Surface water	Sediment Extractables		Sediment combustion + filter paper	Total recovery
		Methanol	Carbon Dioxide		Methanol	Acidic acetone		
0	1	-	-	20.09	81.60	5.72	0.06	107.48
	6	-	-	22.02	66.34	4.12	0.05	92.52
	mean	-	-	21.06	73.97	4.92	0.06	10-
7	4	0.21	0.15	4.66	88.33	6.52	1.29	101.16
	5	0.13	0.10	3.35	87.38	5.38	3.28	99.60
	mean	0.17	0.12	4.00	87.85	5.95	2.28	100.38
14	15	0.06	-	3.13	87.86	3.79	3.24	98.09
	16	-	0.08	3.78	86.65	4.64	3.25	98.40
	mean	0.03	0.04	3.45	87.26	4.22	3.25	98.24
30	7	-	0.43	2.51	81.22	8.82	3.32	96.29
	8	-	0.48	2.66	80.69	9.31	3.12	96.27
	mean	-	0.46	2.59	80.95	9.06	3.22	96.28
60	14	0.02	-	2.22	87.31	11.44	5.80	106.78
	17	0.03	0.04	2.12	86.28	11.98	5.57	106.01
	mean	0.02	0.02	2.17	86.79	11.71	5.68	106.40
90	9	0.05	-	2.36	79.43	8.85	5.52	96.21
	13	0.01	-	2.44	79.42	7.68	5.68	95.23
	mean	0.03	-	2.40	79.42	8.27	5.60	95.72
120	10	0.03	-	2.48	77.85	12.80	6.38	99.54
	11	-	-	1.94	74.42	12.36	5.90	94.62
	mean	0.01	-	2.21	76.14	12.58	6.14	97.08
180	12	0.94	2.76	3.78	79.59	5.35	4.54	96.96
	18	1.04	1.50	3.86	79.39	5.48	4.81	96.06
	mean	0.99	2.13	3.82	79.49	5.41	4.67	96.51
270	2	0.46	-	1.81	88.17	6.65	7.16	104.26
	3	0.85	-	1.29	88.32	8.59	7.66	106.71
	mean	0.66	-	1.55	88.24	7.62	7.41	105.48
365	25	0.34	1.07	1.73	76.11	14.76	2.12	96.12
	26	0.17	1.18	2.00	74.50	15.26	2.18	95.28
	mean	0.25	1.12	1.86	75.30	15.01	2.15	95.70
							Overall mean recovery	**99.18**

Table A7.1.2.2.2-13 The Composition of Radioactivity from methanol extracts, acidic acetone extracts and pond water extracts

[% recovery of applied dose (ppm)]

Metabolites	0	7 days	14 days	30 days	60 days	90 days	120 days	180 days	270 days	365 days
Fipronil	99.46 (0.0497)	80.13 (0.0401)	50.13 (0.0251)	17.33 (0.0087)	4.07 (0.0020)	1.82 (0.0009)			0.62 (0.0003)	
XXXX		17.67 (0.0088)	42.78 (0.0214)	73.99 (0.00370)	78.89 (0.0394)	80.80 (0.0404)	88.72 (0.0444)	83.52 (0.0418)	86.89 (0.0434)	82.58 (0.013)
XXXX	0.49 (0.0003)		0.27 (0.0001)	0.92 (0.0005)	11.09* (0.0055)					
XXXX					0.52 (0.0003)	0.48 (0.0002)		1.38 (0.0007)	4.04 (0.0020)	7.73 (0.0039)
XXXX			1.57 (0.0008)						0.33 (0.0002)	
Component 1			0.18 (0.0001)	0.36 (0.0002)	3.93 (0.0020)	4.58 (0.0023)			0.73 (0.0004)	
Component 2									3.24 (0.0016)	

*= seen primarily in only one sample

Table A7.1.2.2.2-14 The Composition of Radioactivity from Pond Water Extracts

Metabolites	Percent Recovery of the Applied Quantity (ppm) at Indicated Samping time (Day)									
	0	7 days	14 days	30 days	60 days	90 days	120 days	180 days	270 days	365 days
Fipronil	20.57 (0.0103)	4.00 (0.0020)	3.00 (0.0015)	1.11 (0.0006)						
XXXX				0.69 (0.0003)						
XXXX	0.49 (0.0003)		0.27 (0.0001)	0.43 (0.0002)						
XXXX										
XXXX										
Component 1			0.18 (0.0001)	0.36 (0.0002)						
Component 2										

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2009.
Materials and methods	Agree with the applicant's version.
Conclusion	*According to the French RMS for PPP (Draft assessment report April 2004):* Total recovery ranged from 95.7% to 106.4% of TAR throughout the study. Volatiles were < 3.1% and bound residues < 7.4%. Fipronil was no longer detected in water after 30 d; it was adsorbed on the sediment and decreased slowly (1.77% at 90 d). XXXX was the main metabolite in sediment; it increased from 17.67% of the applied RA at 7 days to 78.89% at 60 days and then remained at a level of 80% to 87% thereafter (max. 88.7% in sediment at 120 d). Minor metabolites were identified by HPLC: XXXX, XXXX, and 2 unknowns, each of them < 4%. The degradation of fipronil in these aerobic aquatic conditions followed a first order kinetic with a half-life of 14.5 days for the whole system (R2 = 0.98), calculated by the notifier.
Reliability	2.
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.1.2.2.2/03** Ayliffe J.M. [14C]-Fipronil degradation and retention in two water/sediment systems XXXX February 1998. (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EEC 95/36; SETAC	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Fipronil	
3.1.1	Lot/Batch number	GHS 9321	
3.1.2	Specification	Not given	
3.1.3	Purity	>98% radiochemical purity	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	[^{14}C]-Fipronil with a specific activity of 26.5 mCi.mmol^{-1} with a radiochemical purity greater than 98% was used in this study. Chemical name (IUPAC): (+)-5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethanesulfinyl-1H-pyrazole-3-carbonitrile.	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

	The degradation and retention of [14C]-Fipronil was studied in two water/sediment systems. The two sediments were covered with water with sediment to water ratios of 1:3.1 (Ongar) and 1:3.3 (Manningtree) on a weight basis. Samples of sediment and water were collected fresh from Aldhams Farm, Manningtree, Essex and from Ongar, Essex and stored at 4°C for one week prior to use. Samples were incubated in glass jars containing a sediment depth of approximately 4 cm. The sediment was covered with water to an approximate depth of 11 cm, thus the sediment to water ratio 1:3.1 for Ongar and 1:3.3 for Manningtree on a weight basis.. The properties of the sediment and water are given in Tables A7.1.2.2.2-15 and A7.1.2.2.2-16 The water/sediment systems were acclimatised for 28 days and 91.85 µg of [^{14}C]-Fipronil was applied in 130 µl of acetonitrile to the water phase of each vessel. At each sampling time duplicate glass jars were taken for analysis. The surface water was subjected to a SPE cartridge and eluted with acetonitrile and acetonitrile/water by shaking and some samples were soxhlet extracted with acetonitrile/water. Aliquots of the solvent extracts were concentrated prior to chromatographic analysis by radio-HPLC, with selected samples analysed by radio-TLC, structural confirmation was obtained by LC/MS.	
5.2 Results and discussion	A good recovery of radioactivity was obtained for both systems with an overall mean recovery of 94.4% for the Ongar system and 96.1% for the Manningtree system. Tables A7.1.2.2.2-17 and A7.1.2.2.2-18. There was a gradual transfer of radioactivity from the water phase to the sediment. The unextractable residues remained low throughout the study, generally <5%. Very little volatile radioactivity was evolved (<1%) in both systems. There was a steady decline of Fipronil in both systems with less than 1% remaining after 121 days in the Ongar system and 12% after 121 days in the Manningtree system. Fipronil was the major component present in the surface water, no metabolite exceeded 10%. The minor metabolites in the surface water were XXXX, XXXX and XXXX. In the sediment XXXX the reduced metabolite was the major metabolite present, accounting for up to 80% of the applied radioactivity in both the Ongar and Manningtree systems. There was no other major metabolite in the sediment, though traces of XXXX and XXXX were observed. The DT_{50} of fipronil in the water phase was 5.85 to 13.41 days with the DT_{90} ranging from 34.69 to 46.83 days using a two-compartment model. The DT_{50} and DT_{90} values for the sediment and complete system are given in table A7.1.2.2.2-23.	

Section 7.1.2.2.2 **Annex Point IIIA, XII.2.1**	**Water/sediment degradation study**	

5.3	**Conclusion**	Fipronil was readily degraded in aerobic water with anaerobic sediment systems with DT_{50} values in the water of less than 14 days and in the total system less than 35 days. Fipronil was the only major component found in the water. It rapidly transferred to the sediment (up to 20 to 40% of applied) and was reduced to XXXX which was the major metabolite in the sediment, which undergoes further degradation. Thus Fipronil is unlikely to persist in the aquatic environment.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Table A7.1.2.2.2-15 Physico-Chemical Properties of the Sediments

System Reference	97/07 (Ongar)	97/08 (Manningtree)
Grid Reference of Location	TL566048	TM100302
Collection Date	13 March 1997	14 March 1997
Particle Size Distribution (µm)	%	%
500-2000	5.20	19.25
250-500	24.17	13.72
106-250	14.36	14.62
63-106	4.56	4.42
20-63	13.27	30.21
2-20	13.44	10.34
<2	25.00	7.44
Total	100.00	100.00
Textural classification		
ADAS Classification	Clay loam	Sandy loam
Sand (63-2000 µm)	48.29	52.01
Silt (2-63 µm)	26.71	40.55
Clay (<2 µm)	25	7.44
USDA Classification	Sandy Clay loam	Sandy Loam
Sand (50-2000 µm)	52.30	61.14
Silt (2-50 µm)	22.70	31.42
Clay (<2 µm)	25.00	7.44
Organic Carbon (%)	2.3	2.7
Organic Matter (%)	4.0	4.6
Cation Exchange Capacity (mEq/100g)	63.6	8.15
pH: Water	8.2	6.8
1M KCl	7.7	6.6
0.01M $CaCl_2$	7.6	6.5
Total nitrogen (mg/kg)	1911.1	2086.0
Total phosphorous (mg/kg)	953.7	615.6
Start of study (µg C g^{-1})	143	258
Finish of study 121 days (µg C g^{-1})	140	183

Table A7.1.2.2.2-16 Characterisation of Surface Water (at time of collection)

Sediment Aquatic System	97/07	97/08
Source	River Roding Boarded Barns Farm, Ongar, Essex, UK	Stream Aldhams Farm, Manningtree, Essex, UK
Total nitrogen (ppm)	3.6	2.0
Total phosphorous (ppm)	0.5	0.8
Total Organic Carbon (ppm)	5.0	3.2
Water Hardness ($mg.L^{-1}$ as $CaCO_3$)	465	463
Temperature (°C)	9	8
pH	8.20	6.83
Redox Potential (mV)	+469	+306
Oxygen content just below surface (%)	95	60
Oxygen content sediment level (%)	86	-

Table A7.1.2.2.2-17 Recovery of Radioactivity from Ongar Aquatic Sediment System Following Application of [^{14}C]-Fipronil to the Water Surface

Sampling Time	% of Applied Radioactivity					
	Water	Solvent Extract	Soxhlet Extract	Volatiles	Unextracted	Total
0	100	n.a.	n.a.	n.a.	n.a.	100
0.5 Hour	84.91	7.63	n.a.	n.a.	0.87	93.41
	87.90	5.60	n.a.	n.a.	0.92	94.41
	86.41	6.61	n.a.	n.a.	0.90	93.91
6 Hours	88.45	1.59	n.a.	0.02	0.33	90.40
	89.95	2.00	n.a.	0.02	0.32	92.29
	89.20	1.80	n.a.	0.02	0.32	91.34
1 Day	90.10	1.40	n.a.	0.02	0.10	91.61
	88.07	3.61	n.a.	0.02	0.69	92.39
	89.08	2.50	n.a.	0.02	0.40	92.00
2 Days	86.65	5.58	n.a.	0.02	0.64	92.89
	89.49	2.97	n.a.	0.02	0.34	92.81
	88.07	4.27	n.a.	0.02	0.49	92.85
7 Days	59.03	31.71	n.a.	0.01	4.14	94.89
	70.32	23.14	n.a.	0.01	2.06	95.53
	64.68	27.42	n.a.	0.01	3.10	95.21
14 Days	51.19	38.22	n.a.	0.01	4.76	94.18
	51.21	38.58	n.a.	0.02	4.88	94.68
	51.20	38.40	n.a.	0.01	4.82	94.43
29 Days	29.83	55.68	7.88	0.05	1.58	95.02
	32.59	57.06	6.21	0.05	0.92	96.84
	31.21	56.37	7.05	0.05	1.25	95.93
58 Days	19.94	63.55	10.87	0.10	1.84	96.30
	20.68	63.21	9.84	0.08	1.58	95.39
	20.31	63.38	10.36	0.09	1.71	95.84
93 Days	14.70	69.85	10.32	0.15	2.33	97.34
	13.86	68.86	11.29	0.14	1.95	96.09
	14.28	69.36	10.80	0.14	2.14	96.72
121 Days	12.91	70.84	9.00	0.14	2.38	95.27
	11.91	71.88	10.17	0.19	2.31	96.46
	12.41	71.36	9.59	0.17	2.34	95.87
					Overall mean	**94.30**

0.00 signifies below detection limit in this sample
n.a. signifies that sample was not extracted during analysis

Table A7.1.2.2.2-18 Recovery of Radioactivity from Manningtree Aquatic Sediment System Following Application of [^{14}C]-Fipronil to the Water Surface

Sampling Time	% of Applied Radioactivity					
	Water	Solvent Extract	Soxhlet Extract	Volatiles	Unextracted	Total
0	100	n.a.	n.a.	n.a.	n.a.	100
0.5 Hour	87.73	5.57	n.a.	n.a.	1.16	94.46
	88.99	6.76	n.a.	n.a.	1.42	97.17
	88.36	6.16	n.a.	n.a.	0.86	95.82
6 Hours	80.73	10.26	n.a.	0.02	1.68	92.69
	84.15	7.95	n.a.	0.02	1.79	93.91
	82.44	9.10	n.a.	0.02	1.16	93.30
1 Day	84.86	7.90	n.a.	0.02	1.36	94.14
	84.72	4.70	n.a.	0.02	0.84	90.27
	84.79	6.30	n.a.	0.02	0.73	92.20
2 Days	65.10	29.68	n.a.	0.03	1.82	96.63
	52.63	41.76	n.a.	0.02	2.43	96.84
	58.87	35.72	n.a.	0.02	1.42	96.73
7 Days	40.60	48.30	n.a.	0.01	6.87	95.78
	48.46	43.09	n.a.	0.01	4.90	96.45
	44.53	45.70	n.a.	0.01	5.88	96.12
14 Days	31.35	51.43	11.12	0.00	1.10	95.00
	30.44	52.98	11.36	0.01	1.16	95.94
	30.90	52.21	11.24	0.00	1.13	95.47
29 Days	16.04	71.95	9.28	0.01	2.11	99.39
	13.64	44.51	36.74	0.01	2.25	97.14
	14.84	58.23	23.01	0.01	2.18	98.26
58 Days	2.41	76.92	14.10	0.03	2.64	96.10
	9.84	69.83	18.47	0.04	2.12	100.31
	6.12	73.38	16.29	0.03	2.38	98.20
93 Days	5.17	59.56	25.80	0.04	2.91	93.48
	3.24	64.99	30.06	0.04	3.37	101.70
	4.21	62.27	27.93	0.04	3.14	97.59
121 Days	2.35	71.69	19.34	0.05	3.37	96.80
	2.58	73.67	17.38	0.04	3.31	96.98
	2.47	72.68	18.36	0.05	3.34	96.89
					Overall mean	**95.88**

0.00 signifies below detection limit in this sample
n.a. signifies that sample was not extracted during analysis

Table A7.1.2.2.2-19 Composition of Radioactivity in the Surface Water (Ongar)

Sampling Time	% of Applied Radioactivity					
	Total	**Fipronil**	XXXX	XXXX	XXXX	**Component RT<5mins**
0.5 Hour	84.91	84.91	n.d.	n.d.	n.d.	n.d.
	87.90	87.90	n.d.	n.d.	n.d.	n.d.
	86.41	86.41	n.d.	n.d.	n.d.	n.d.
6 Hours	88.45	88.45	n.d.	n.d.	n.d.	n.d.
	89.95	89.95	n.d.	n.d.	n.d.	n.d.
	89.20	89.20	n.d.	n.d.	n.d.	n.d.
1 Day	90.10	90.10	n.d.	n.d.	n.d.	n.d.
	88.07	88.07	n.d.	n.d.	n.d.	n.d.
	89.08	89.08	n.d.	n.d.	n.d.	n.d.
2 Days	86.65	86.65	n.d.	n.d.	n.d.	n.d.
	89.49	89.49	n.d.	n.d.	n.d.	n.d.
	88.07	88.07	n.d.	n.d.	n.d.	n.d.
7 Days	59.03	59.03	n.d.	n.d.	n.d.	n.d.
	70.32	68.62	n.d.	n.d.	n.d.	1.40
	64.68	63.83	n.d.	n.d.	n.d.	0.85
14 Days	51.19	46.57	4.62	n.d.	n.d.	n.d.
	51.21	46.49	4.72	n.d.	n.d.	n.d.
	51.20	46.53	4.67	n.d.	n.d.	n.d.
29 Days	29.83	15.94	9.02	1.38	1.51	1.98
	32.59	22.36	8.25	0.73	1.25	n.d.
	31.21	19.15	8.64	1.06	1.38	0.99
58 Days	19.94	5.73	8.19	1.77	1.90	2.36
	20.68	10.03	6.62	2.04	1.99	n.d.
	20.31	7.88	7.41	1.90	1.94	1.18
93 Days	14.70	1.99	9.33	n.d.	1.83	1.54
	13.86	1.74	8.38	n.d.	2.53	1.21
	14.28	1.87	8.86	n.d.	2.18	1.38
121 Days	12.91	0.43	7.35	0.41	3.01	1.71
	11.91	0.54	6.32	0.55	3.13	1.37
	12.41	0.48	6.83	0.48	3.07	1.54

Two flasks were analysed at each timepoint
n.d. = not detected
RT = retention time

Table A7.1.2.2.2-20 Composition of Radioactivity in the Sediment (Ongar)

	% of Applied Radioactivity					
Sampling Time	**Total**	**Fipronil**	XXXX	XXXX	XXXX	**Component RT<5mins**
0.5 Hour	7.63	7.63	n.d.	n.d.	n.d.	n.d.
	5.60	n.a.	n.a.	n.a.	n.a.	n.a.
	6.61	6.61	n.d.	n.d.	n.d.	n.d.
6 Hours	1.59	n.a.	n.a.	n.a.	n.a.	n.a.
	2.00	n.a.	n.a.	n.a.	n.a.	n.a.
	1.80	n.a.	n.a.	n.a.	n.a.	n.a.
1 Day	1.40	n.a.	n.a.	n.a.	n.a.	n.a.
	3.61	n.a.	n.a.	n.a.	n.a.	n.a.
	2.50	n.a.	n.a.	n.a.	n.a.	n.a.
2 Days	5.58	5.58	n.d.	n.d.	n.d.	n.d.
	2.97	n.a.	n.a.	n.a.	n.a.	n.a.
	4.27	4.27	n.d.	n.d.	n.d.	n.d.
7 Days	31.71	18.71	13.00	n.d.	n.d.	n.d.
	23.14	14.02	9.12	n.d.	n.d.	n.d.
	27.42	16.37	11.06	n.d.	n.d.	n.d.
14 Days	38.22	12.79	25.43	n.d.	n.d.	n.d.
	38.58	17.06	21.52	n.d.	n.d.	n.d.
	38.40	14.93	23.47	n.d.	n.d.	n.d.
29 Days	63.56	12.76	50.79	n.d.	n.d.	n.d.
	63.28	14.26	49.01	n.d.	n.d.	n.d.
	63.42	13.51	49.90	n.d.	n.d.	n.d.
58 Days	74.42	7.43	66.99	n.d.	n.d.	n.d.
	73.05	7.62	65.43	n.d.	n.d.	n.d.
	73.73	7.52	66.21	n.d.	n.d.	n.d.
93 Days	80.17	n.d.	80.17	n.d.	n.d.	n.d.
	80.15	0.26	78.78	0.23	0.87	n.d.
	80.16	0.13	79.48	0.11	0.44	n.d.
121 Days	79.84	n.d.	79.84	n.d.	n.d.	n.d.
	82.06	n.d.	82.06	n.d.	n.d.	n.d.
	80.95	n.d.	80.95	n.d.	n.d.	n.d.

Two flasks were analysed at each timepoint
n.a. = not analysed
n.d. = not detected
RT = retention time

Table A7.1.2.2.2-21 Composition of Radioactivity in the Surface Water (Manningtree)

	% of Applied Radioactivity					
Sampling Time	**Total**	**Fipronil**	**XXXX**	**XXXX**	**XXXX**	**Component RT<5mins**
0.5 Hour	87.73	87.73	n.d.	n.d.	n.d.	n.d.
	88.99	88.99	n.d.	n.d.	n.d.	n.d.
	88.36	88.36	n.d.	n.d.	n.d.	n.d.
6 Hours	80.73	80.73	n.d.	n.d.	n.d.	n.d.
	84.15	84.15	n.d.	n.d.	n.d.	n.d.
	82.44	82.44	n.d.	n.d.	n.d.	n.d.
1 Day	84.86	84.86	n.d.	n.d.	n.d.	n.d.
	84.72	84.72	n.d.	n.d.	n.d.	n.d.
	84.79	84.79	n.d.	n.d.	n.d.	n.d.
2 Days	65.10	65.10	n.d.	n.d.	n.d.	n.d.
	52.63	52.63	n.d.	n.d.	n.d.	n.d.
	58.87	58.87	n.d.	n.d.	n.d.	n.d.
7 Days	40.60	40.60	n.d.	n.d.	n.d.	n.d.
	48.46	48.46	n.d.	n.d.	n.d.	n.d.
	44.53	44.53	n.d.	n.d.	n.d.	n.d.
14 Days	31.35	31.35	n.d.	n.d.	n.d.	n.d.
	30.44	30.44	n.d.	n.d.	n.d.	n.d.
	30.90	30.90	n.d.	n.d.	n.d.	n.d.
29 Days	16.04	10.49	3.91	1.63	n.d.	n.d.
	13.64	10.90	0.95	1.79	n.d.	n.d.
	14.84	10.70	2.43	1.71	n.d.	n.d.
58 Days	2.41	n.a.	n.a.	n.a.	n.a.	n.a.
	9.84	5.52	1.56	1.80	0.97	n.d.
	6.12	3.43	0.97	1.12	0.60	n.d.
93 Days	5.17	0.73	0.74	0.82	2.05	0.82
	3.24	n.d.	0.72	0.69	1.05	0.78
	4.21	0.37	0.73	0.76	1.55	0.80
121 Days	2.35	0.25	0.84	n.d.	0.80	0.46
	2.58	0.60	0.60	0.34	0.62	0.42
	2.47	0.42	0.72	0.17	0.71	0.44

Two flasks were analysed at each timepoint
n.a. = not analysed
n.d. = not detected
RT = retention time

Table A7.1.2.2.2-22 Composition of Radioactivity in the Sediment (Manningtree)

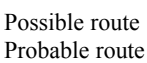
Sampling Time	% of Applied Radioactivity					
	Total	Fipronil	XXXX	XXXX	XXXX	Component RT<5mins
0.5 Hour	5.57	n.a.	n.a.	n.a.	n.a.	n.a.
	6.76	6.76	n.d.	n.d.	n.d.	n.d.
	6.16	6.16	n.d.	n.d.	n.d.	n.d.
6 Hours	10.26	10.26	n.d.	n.d.	n.d.	n.d.
	7.95	n.a.	n.a.	n.a.	n.a.	n.a.
	9.10	9.10	n.d.	n.d.	n.d.	n.d.
1 Day	7.90	7.90	n.d.	n.d.	n.d.	n.d.
	4.70	n.a.	n.a.	n.a.	n.a.	n.a.
	6.30	6.30	n.d.	n.d.	n.d.	n.d.
2 Days	29.68	29.68	n.d.	n.d.	n.d.	n.d.
	41.76	41.76	n.d.	n.d.	n.d.	n.d.
	35.72	35.72	n.d.	n.d.	n.d.	n.d.
7 Days	48.30	35.61	12.69	n.d.	n.d.	n.d.
	43.09	33.10	10.00	n.d.	n.d.	n.d.
	45.70	34.35	11.34	n.d.	n.d.	n.d.
14 Days	62.54	36.02	26.52	n.d.	n.d.	n.d.
	64.34	45.44	18.90	n.d.	n.d.	n.d.
	63.44	40.73	22.71	n.d.	n.d.	n.d.
29 Days	81.23	34.91	46.32	n.d.	n.d.	n.d.
	81.24	40.67	40.57	n.d.	n.d.	n.d.
	81.24	37.79	43.45	n.d.	n.d.	n.d.
58 Days	91.02	35.99	55.03	n.d.	n.d.	n.d.
	88.31	20.73	67.58	n.d.	n.d.	n.d.
	89.66	28.36	61.30	n.d.	n.d.	n.d.
93 Days	85.36	9.95	69.71	5.39	0.30	n.d.
	95.05	9.64	77.60	7.82	n.d.	n.d.
	90.20	9.80	73.65	6.60	0.15	n.d.
121 Days	91.03	1.55	89.48	n.d.	n.d.	n.d.
	91.05	21.35	69.70	n.d.	n.d.	n.d.
	91.04	11.45	79.59	n.d.	n.d.	n.d.

Two flasks were analysed at each timepoint
n.a. = not analysed
n.d. = not detected
RT = retention time

Table A7.1.2.2.2-23 Compartment model best-fit values

System	Fipronil		
	Phase	DT_{50}	DT_{90}
Ongar	Water	13.41 days	46.83 days
	Sediment	47.54 days	97.63 days
	Complete System	21.20 days	64.76 days
Manningtree	Water	5.85 days	34.69 days
	Sediment	74.80 days	161.63 days
	Complete System	31.68 days	121.59 days

Figure A7.1.2.2.2-2 Proposed Degradation Pathway for Fipronil in Water/Sediment Systems



	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2009.
Materials and methods	Agree with the applicant's version.
Conclusion	*According to the French RMS for PPP (Draft assessment report April 2004):* There was a good recovery of radioactivity for both systems, with an overall mean recovery of 94.4% for the Ongar system and 96.1% for the Manningtree system (see table 8.4.3.2 - 4). Volatiles were quite low, never exceeding 0.17%. The amount of bound residue on sediment was low (max. 5.9%) Fipronil was rapidly adsorbed on sediment (max. 40.7% at 14 d) and then degraded ; the main metabolite was XXXX ; it reached 80% at 121 d and no decrease could be observed during the experiment ; a few amount of this metabolite (max. 8.8% at 93 d) was detected in water. Other metabolites, XXXX and XXXX (from hydrolysis of XXXX) were detected, mainly in water, and none exceeded 2%. The rate of degradation of fipronil, given in table 8.4.3.2 - 6 were estimated by the notifier with different models. Timme et Fresh model gave the poorest adjustment. The RMS agrees with the calculation performed considering a linear first order degradation and proposes to consider these values of DT50, 14.2 and 16.2 days in water, 16.4 and 35.6 days in the whole system. It was impossible to estimate DT50 of XXXX in this water sediment system, as no decrease could be observed during the experiment. As the OC content of the 2 sediments studied were not different enough, another study was conducted, in 2002.
Reliability	2.
Acceptability	Acceptable.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.3 **Annex Point IIA, VII.7.7**	**Adsorption/desorption test**	

	1. REFERENCE	**Official use only**

Section 7.1.3 **Annex Point IIA, VII.7.7**	**Adsorption/desorption test**	

1.1	**Reference**	**A7.1.3/01** Godward, P.J., Austin, D.J. and Quarmby, D.L. MB46030-^{14}C Adsorption/desorption on five soils (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: USEPA 163-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	Batch IHR 1465	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	99.3 to 99.4%	
3.1.4	Further relevant properties	Specific activity 19.62 mCi.mmol^{-1}	
3.1.5	Method of analysis	Radiochemical (LSC)	
3.2	**Degradation products**	No	
3.2.1	Method of analysis for degradation products	Not applicable	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Soil types**	Speyer 2.2 (Germany) sandy loam (UK) loam (UK) sandy-clay loam-1 (France) sandy-clay loam-2 (France)	
3.5	**Testing procedure**		
3.5.1	Test systems	2.5g air dried soil was weighed into pre-weighed, numbered glass tubes (30ml capacity) fitted with glass stoppers.	

Section 7.1.3 **Annex Point IIA, VII.7.7**	**Adsorption/desorption test**	
3.5.2 Test solution and Test Conditions	1, 0.2, 0.05 and 0.01 µg/ml in solution containing 0.005M aqueous calcium chloride with a total volume of 10 ml. The experiment was carried out at ambient temperature (approximately 20°C).	
3.6 Test performance		
3.6.1 Preliminary test	Purity of test substance was tested by TLC.	
3.6.2 Screening test: Adsorption	A test was carried out to determine the time required for equilibrium concentrations during adsorption phase.	
3.6.3 Screening test: Desorption	A test was carried out to determine the time required for equilibrium concentrations during first desorption phase.	
3.6.4 HPLC-method	HPLC chromatograms of adsorbate and desorbate were obtained using a Waters HLPC system equipped with a Spherisorb ODS2 column (5µm, 25 cm x 4.6 mm i.d) and a Water 484 UV detector at 280 nm or a Raytest Ramona 5 for ^{14}C detection.	
3.6.5 Other test	Advanced test according to USEPA 163-1	
	4. RESULTS	
4.1 Preliminary test	The test substance showed a high radiopurity (> 99%).	
4.2 Screening test: Adsorption	It was concluded from the screening test that a shaking time of 24 h was required for adsorbtion.	
4.3 Screening test: Desorption	It was concluded from the screening test that a shaking time of 1 h was required for desorption.	
4.4 Calculations		
4.4.1 Ka, Kd	See tables A7.1.3-2 to 7.1.3-8 Mean Ka 11.86 mL/g Mean Kd 13.03 mL/g (first cycle)	
4.4.2 Ka_{oc} , Kd_{oc}	See tables A7.1.3-2 to 7.1.3-8 Mean Ka_{oc} 727 mL/g; 1/n = 0.951 Mean Kd_{oc} 949 mL/g; 1/n = 0.942 (first cycle)	
4.5 Degradation product(s)	Fipronil was stable under the test conditions	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	[^{14}C]-Fipronil of radiochemical purity 99.3/99.4%; specific activity 19.62 $mCi.mmol^{-1.}$ The soil adsorption/desorption properties of [^{14}C]-Fipronil, have been investigated using five European soil types using the slurry technique. The soils tested were Speyer 2.2 (Germany), sandy loam (UK), loam (UK), sandy-clay loam-1 (France), sandy-clay loam-2 (France).	

Section 7.1.3 **Annex Point IIA, VII.7.7**	**Adsorption/desorption test**	
	Characteristics of the soils are given in Table A7.1.3-1. Each soil was allowed to air dry to constant weight and sieved through a 2mm screen before use. The concentrations were 1, 0.2, 0.05 and 0.01 µg/ml in solution containing 0.005M aqueous calcium chloride with a total volume of 10 ml. Before each batch of sorptions, the concentration of each dosing solution was checked by using liquid scintillation counting (LSC) to determine the radiocarbon content. Adsorption cycles were carried out in duplicate on each soil type at ambient temperature (approximately 20°C). Air dried soil (2.5 g on an oven dried basis) was weighed, treated followed by continuously shaking for approximately 24 hours at a rate sufficient to maintain suspension of the soils. At the end of this period the tubes were centrifuged at 500g for 10 minutes. The supernatants adsorbents were decanted into vials and triplicate aliquots (0.05, 0.25 or 1ml) were taken for radioassay. After removing the supernatants, 0.005M aqueous calcium chloride solution (10ml) was added to each tube and shaken to suspend the soil for approximately 1 hour. The tubes were centrifuged as above, the supernatants removed and their weight recorded. Triplicate aliquots (0.05 to 3.0ml) were taken for radioassay. The entire procedure was repeated four times, giving a total of five desorbates. After the fifth desorption cycle, the soil residues were extracted with acetonitrile (approximately 10ml), shaking for 15 minutes and centrifuged as before. Triplicate aliquots (0.05, 0.25 or 1.0ml) of the acetonitrile extracts were assayed. After air-drying, the extracted soil residues were weighed, combusted and assayed for radioactivity.	X
5.2 Results and discussion	The amount of radioactivity in each adsorbate, desorbate, acetonitrile extract and soil residue was calculated and summed to obtain the total recovered radioactivity. This was expressed as a percentage of the originally applied radioactivity, these recoveries are shown in Table A7.1.3-2. The K_{OC} values were calculated using the Freundlich equation for adsorption and desorption. Results for adsorption and desorption cycles are given in Tables A7.1.3-3 to 8. The value of K increased with increasing organic carbon content of the soil suggesting that more fipronil was adsorbed. The Freundlich desorption constants increased with the increasing desorption cycles, the results suggest that the adsorption was reversible with similar processes involved in the desorption as the adsorption	
5.2.1 Adsorbed a.s.[%]	Not given in report	
5.2.2 K_a	The adsorption constants (K) obtained ranged from 4.19 in the UK sandy loam to 20.69 in the UK loam (mean 11.86).	
5.2.3 K_d	The desorption constants (K) obtained ranged from 7.25 in the UK sandy loam to 21.51 in the UK loam (mean 13.03).	
5.2.4 Ka_{oc}	The K_{OC} values obtained ranged from 427 to 1248 with a mean of 727.	

Section 7.1.3 **Annex Point IIA, VII.7.7**	**Adsorption/desorption test**	

5.2.5 Ka/Kd	Comparison of the isotherms derived from adsorption and desorption data indicates that the processes involved in adsorption and desorption are similar.	
5.2.6 Degradation products (% of a.s.)	Fipronil was stable under the test conditions.	
5.3 Conclusion	Comparison of the isotherms derived from adsorption and desorption data indicates that the processes involved in adsorption and desorption are similar. The results indicate that fipronil is unlikely to demonstrate significant mobility in soil due to its relatively high sorption t soil. According to McCall's designation, fipronil would be expected to show medium to low mobility	
5.3.1 Reliability	1	**X**
5.3.2 Deficiencies	None	

Table A7.1.3-1 Classification and physico-chemical properties of soils used as adsorbents

	Soil 1	**Soil 2**	**Soil 3**	**Soil 4**	**Soil 5**
Location	Speyer 2.2 (D)	Manningtree (UK)	Manningtree (UK)	Chazay (F)	Chazay (F)
Soil Designation	91/6	91/8	91/10	91/11	91/12
Clay (%)	8	12	25	24	34
Silt (%)	9	11	29	18	19
Sand (%)	83	77	46	58	47
USDA Classification	Loamy sand	Sandy loam	Loam	Sandy clay loam 1	Sandy clay loam 2
pH	6.3	6.1	6.9	6.2	6.3
Organic Matter (%)	5.70	0.57	7.23	1.98	2.71
Water Content of air dried soils (%)	1.14	0.60	3.99	1.36	2.59
Cation Exchange Capacity (mEq/100g)	10.76	7.15	36.51	12.57	20.41
Moisture holding capacity (%) 1/3 bar 0.1 bar	 10.7 13.4	 9.2 NA	 31.3 NA	 21.0 30.9	 23.6 35.8
Bulk density (g/cm^3)	1.46	1.59	1.30	1.55	1.31
Biomass (μg^{-1} dry weight)	471	58	1420	268	700
Microbial Count (g^{-1} dry					

weight)					
Bacteria	2.1×10^6	$<1 \times 10^5$	7.6×10^6	9.0×10^6	7.6×10^6
Actinomycetes	1.4×10^6	2.8×10^6	1.4×10^6	1.1×10^6	2.1×10^6
Fungi	6.4×10^3	1.0×10^2	2.7×10^3	3.8×10^3	2.3×10^3

Table A7.1.3-2 Distribution and recovery of radioactivity (%)

Concentration (µg/ml)	Loamy Sand (91/6)	Sandy Loam (91/8)	Loam (91/10)	Sandy Clay Loam (91/11)	Sandy Clay Loam (91/12)
	%	%	%	%	%
1.0	100.5 99.10	93.41 92.93	106.34 102.59	95.30 91.66	97.80 95.99
0.2	94.11 89.07	91.78 90.93	100.68 100.86	92.59 91.94	94.00 91.73
0.05	87.57 89.98	91.25 89.64	97.43 97.79	92.05 91.22	95.43 94.46
0.01	101.31 86.50	91.38 93.50	93.74 93.66	90.32 91.18	94.28 89.45
Mean	93.52	91.85	99.14	92.03	94.14

Table A7.1.3-3 Freudlich adsorption constants for Fipronil

Soil type	% organic carbon	Ka	1/n	Koc
~~Speyer 2.2~~ **Loamy sand**	3.35	14.32	0.947	427
Sandy Loam	0.34	4.19	0.950	1248
Loam	4.25	20.69	0.938	486
Sandy-clay-loam-1	1.16	9.32	0.969	800
Sandy-clay-loam-2	1.59	~~10.78~~ **10.73**	0.949	673

Mean Koc = 727

X

Table A7.1.3-4 Freudlich desorption constants for Fipronil (1st cycle)

Soil type	% organic carbon	Kdes	1/n	Koc_{des}
~~Speyer 2.2~~ **Loamy sand**	3.35	13.35	0.905	398
Sandy Loam	0.34	7.25	0.986	2162
Loam	4.25	21.51	0.910	506
Sandy-clay-loam-1	1.16	10.14	0.960	870
Sandy-clay-loam-2	1.59	12.88	0.948	808

Mean Koc = 949

Table A7.1.3-5 Freudlich desorption constants for Fipronil (2nd cycle)

Soil type	% organic carbon	Kdes	1/n	Koc_{des}
Speyer 2.2	3.35	16.71	0.931	498
Sandy Loam	0.34	9.28	0.984	2768
Loam	4.25	19.74	0.873	464
Sandy-clay-loam-1	1.16	10.36	0.938	890
Sandy-clay-loam-2	1.59	12.26	0.920	769

Mean Koc = 1078

Table A7.1.3-6 Freudlich desorption constants for Fipronil (3rd cycle)

Soil type	% organic carbon	Kdes	1/n	Koc_{des}
Speyer 2.2	3.35	20.73	0.918	618
Sandy Loam	0.34	14.65	1.003	4369
Loam	4.25	23.36	0.899	549
Sandy-clay-loam-1	1.16	13.05	0.950	1121
Sandy-clay-loam-2	1.59	13.33	0.928	836

Mean Koc = 1499

Table A7.1.3-7 Freudlich desorption constants for Fipronil (4th cycle)

Soil type	% organic carbon	Kdes	1/n	Koc_{des}
Speyer 2.2	3.35	20.92	0.913	624
Sandy Loam	0.34	19.15	0.997	5711
Loam	4.25	23.16	0.892	544
Sandy-clay-loam-1	1.16	14.05	0.947	1206
Sandy-clay-loam-2	1.59	15.11	0.929	948

Mean Koc = 949

Table A7.1.3-8 Freudlich desorption constants for Fipronil (5th cycle)

Soil type	% organic carbon	Kdes	1/n	Koc_{des}
Speyer 2.2	3.35	22.85	0.925	681
Sandy Loam	0.34	24.50	0.991	7307
Loam	4.25	23.33	0.892	549
Sandy-clay-loam-1	1.16	19.45	0.952	1670
Sandy-clay-loam-2	1.59	15.94	0.926	1000

Mean Koc = 2241

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Applicant's version is acceptable with the following comment: **5.1 Materials and methods** According to OECD Guideline, a solution containing 0.01M aqueous calcium chloride should be used. However, this deviation is not considered as having affected the outcome of the study in a significant way and it is a minor deviation. All 5 soil types don't vary significantly in pH (range is 6.1 to 6.9) and therefore an important demand of the OECD Guideline 106 is not fulfilled. Hence, no statement can be made to the pH-dependency of Fipronil for adsorption/desorption.
Conclusion	Agree with the applicant's version
Reliability	2.
Acceptability	Acceptable
Remarks	Errors in Table A7.1.3-3 were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.1.4 **Further adsorption and desorption studies** **Annex Point IIIA, XII.2.2**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Field study on accumulation in the sediment is not required as less than 70% of non-extractable form in the water/sediment study (see Section 7.1.2.2.2) as indicated in *Guidance on data requirement of active substances and biocidal products, final draft version 4.3.2 October 2000)*	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2 **Annex Point IIIA, XII.1**	**Fate and behaviour in soil**

Section 7.2.1 **Annex Point IIIA, VII.4, XII.1.1**	**Aerobic degradation in soil**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.2.1/01** Waring, A.R. [^{14}C]-M&B 46030 : Aerobic Soil Metabolism (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA and BBA	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Fipronil (purified)	
3.1.1	Lot/Batch number	IHR1465	
3.1.2	Specification	Not given	
3.1.3	Purity	Radiochemical purity 99.5%	
3.1.4	Further relevant properties	Specific radioactivity 44.81 µCi/mg	
3.1.5	Composition of product	Not applicable	
3.2	**Testing procedure**		
3.2.1	Objective	The degradation of [^{14}C]-Fipronil had been investigated in two soils	
3.2.2	Soil Characteristics	Table A7.2.1-1 The microbial biomass was determined both before commencement of the study and at the end. The soils were 2mm sieved, without drying prior to use	

Section 7.2.1 **Annex Point IIIA, VII.4, XII.1.1**	**Aerobic degradation in soil**

3.2.3	Study Design	Aliquots of the soil (50g dry soil equivalent were treated with 0.01mg of fipronil (0.44µCi) in 525µl of acetonitrile. This corresponds to a dose rate equivalent to 200g ai/ha. The soil units were incubated at 25±1°C in the dark. The soil moisture content was adjusted to 75% of the water capacity at 0.33 bar by addition of deionised water, if necessary. Moistened CO_2 free air was drawn through each chamber before being passed through various traps to collect polar and non polar volatiles and $^{14}CO_2$.
3.2.4	Study Method	Duplicate incubation units of each soil type were removed for analysis at intervals of 0, 1, 3, 7, 14, 30, 41, 80, 149, 252 and 336 days after test article application. The total soil sample was soxhlet extracted for 3 hours with acetonitrile (250ml). The radioactivity in acetonitrile extract was quantified by LSC. The acetonitrile extract was concentrated by rotary evaporation prior to chromatographic analysis. The radioactivity in the ethanediol, 2% liquid paraffin in xylene and ethanolamine trapping solutions was quantified monthly or when incubation units were sampled by submitting aliquots to LSC. Traps were recharged with fresh reagent on each sampling occasion. Soil extracts were analysed by HPLC and TLC, the HPLC method was used for quantification, as it was more reliable. Selected extract of soil samples were analysed by gas chromatography/mass spectrometry.
		4. RESULTS
4.1		See Tables 7.2.1-2 to 7 And Figure 7.2.1-1
		5. APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The degradation of [^{14}C]-fipronil applied at a rate of 0.2 mg/kg has been studied in a sandy loam and a sandy soil over a 336 day period under aerobic conditions
5.2	**Results and discussion**	Overall recoveries of applied radioactivity ranged from 92 to 103%. Initially of [^{14}C]-fipronil accounted for >98% of applied radioactivity. Over the study period the amount of parent compound detected declined to 12 to 20% in Manningtree soil and 44 to 46% in Speyer 2.2 soil. Degradation proceeded by hydrolysis to XXXX and oxidation to XXXX. XXXX and XXXX were the main degradation products detected, accounting for up to 38 and 24% of applied radioactivity respectively in Manningtree soil and up to 27 and 14% Speyer 2.2 soil. Smaller quantities of XXXX (<5%) formed by reduction and XXXX (1%) formed by photolysis were also detected in both soil types. XXXX was also detected at <1% in Speyer 2.2 soil. The presence of fipronil, XXXX, XXXX and XXXX was confirmed by GC/MS analysis.

Section 7.2.1 **Annex Point IIIA, VII.4, XII.1.1**	**Aerobic degradation in soil**	
	Six minor unidentified degradation products were detected by HLC in the Manningtree soil and three in the Speyer 2.2 soil each comprising <4% of applied radioactivity. The un-extractable soil residue remained low throughout reaching a maximum of ca 15% in the Manningtree and 6% in the Speyer soils respectively. A proposed metabolic pathway in soil is shown in Section IIIA 7.2.2.1.2 (Aerobic degradation in soil – further studies).	
5.3 Conclusion	The half life of [^{14}C]-Fipronil determined by HPLC in Manningtree and Speyer 2.2 soils under aerobic conditions were 128 and 308 days respectively, degradation proceeded via hydrolysis to XXXX and oxidation to XXXX	X
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A7.2.1-1 Soil Characteristics

Characteristic	**Value**	
Location	**Manningtree**	**Speyer**
Soil Designation	91/9/3	
Particle size Distribution (BBA) >20µm (%) 2 – 20µm (%) <2µm (%) Textural class	 82 11 7 Sand	 93 5 2 Sand
Particle size Distribution (USDA) >50µm (%) 2 – 50µm (%) <2µm (%) Textural class	 56 35 9 Sandy loam	 88 9 3 Sand
pH (KCl) pH (H_2O)	7.1 7.8	5.9 6.1
Organic Carbon	1.0%	1.9%
Organic Matter	1.7%	3.3%
Cation Exchange Capacity mEq/100g	6.4	3.3
Moisture capacity at 33kPa	13.8	14.24
Microbial Biomass µg C/g soil Total viable bacteria (colony forming units)	56.70 (52.86)* 83,000 (6,100,000)*	414.41 (153.71)* 63,000 (1,320,000)*

* Values in parenthesis were determined using the control units at the end of the incubation period

Table A7.2.1-2 The Distribution of Radioactivity in Manningtree Soil

Replicate	Day 0		Day 1		Day 3		Day 7		Day 14		Day 30	
	A	B	A	B	A	B	A	B	A	B	A	B
Soil extract	98.19	100.99	94.03	97.19	92.92	89.99	94.23	97.43	95.14	93.99	89.10	95.62
Ethanediol trap	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2% paraffin in xylene trap	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethanolamine traps	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Unextracted	0.00	0.00	1.49	1.04	2.37	2.12	2.32	1.99	3.13	2.77	4.46	3.70
Total recovery	98.19	100.99	95.52	98.23	95.29	92.11	96.55	99.42	98.27	96.75	93.56	99.32
Mean	99.59		96.88		93.70		97.99		97.52		96.44	

Replicate	Day 41		Day 80		Day 149		Day 252		Day 336	
	A	B	A	B	A	B	A	B	A	B
Soil extract	93.26	91.73	91.93	88.52	88.45	87.96	87.07	86.17	79.73	~~88.11~~ **86.11**
Ethanediol trap	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2% paraffin in xylene trap	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0.03
Ethanolamine traps	0.00	0.00	0.16	0.16	0.23	0.23	0.35	0.35	2.43	2.43
Unextracted	5.24	7.73	7.26	8.85	9.93	10.05	12.03	12.58	15.67	14.52
Total recovery	98.50	99.46	99.36	97.54	98.62	98.25	99.46	99.11	97.86	103.09
Mean	98.98		98.45		98.44		99.29		100.48	

X

Na Not applicable
Overall mean recovery : 97.98%

Table A7.2.1-3 The Distribution of Radioactivity in Speyer 2.2 Soil

Replicate	Day 0		Day 1		Day 3		Day 7		Day 14		Day 30	
	A	B	A	B	A	B	A	B	A	B	A	B
Soil extract	100.73	101.38	101.88	101.84	94.57	96.95	96.48	98.46	93.88	98.15	92.84	9.06
Ethanediol trap	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2% paraffin in xylene trap	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ethanolamine traps	Na	Na	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
Unextracted	0.00	0.00	1.12	1.16	2.75	2.01	2.38	1.82	3.43	3.11	4.94	4.26
Total recovery	100.73	101.38	103.00	103.00	97.32	98.96	98.86	100.28	97.31	101.26	97.80	100.34
Mean	101.06		103.00		~~96.14~~ **98.14**		99.57		99.29		99.07	

X

Replicate	Day 41		Day 80		Day 149		Day 252		Day 336	
	A	B	A	B	A	B	A	B	A	B
Soil extract	94.33	97.74	92.55	~~96.56~~ **96.58**	87.70	95.26	94.60	~~95.82~~ **96.82**	92.90	92.51
Ethanediol trap	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2% paraffin in xylene trap	0.00	0.00	0.01	0.01	0.01	0.01	0.11	0.11	1.18	1.18
Ethanolamine traps	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	1.47	1.47
Unextracted	3.66	4.13	5.26	4.67	6.5	5.61	5.68	4.76	5.54	6.48
Total recovery	98.01	101.89	97.84	101.28	94.25	100.90	100.41	101.71	101.09	101.64
Mean	99.95		99.56		97.57		101.06		101.37	

Na Not applicable
Overall mean recovery : 99.97%

Table A7.2.1-4 Composition of Extractable Radioactivity – Manningtree soil

Time	Day 0			Day 1			Day 3			Day 7			Day 14			Day 30		
Replicate	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**
Compound Fipronil	96.67	100.90	99.79	94.68	97.07	95.88	91.40	85.91	88.66	93.89	93.47	93.68	89.05	90.09	89.57	77.80	84.33	81.07
XXXX											0.81	~~0.81~~ **0.40**	0.98	1.42	1.20	5.73	5.52	5.63
XXXX																		
XXXX										0.61	1.23	0.92	0.91	1.10	1.01	2.16	1.06	1.61
XXXX					0.68	~~0.68~~ **0.34**	1.43	1.44	1.44	2.43	1.98	2.21	3.24	3.09	3.17	4.98	4.97	4.98
Component 1				1.40		~~1.40~~ **0.70**												
Component 2																		
Component 3																		
Component 4																		
Component 5																		
Component 6																		
Total recovery	98.67	100.9	99.79	96.08	97.75	96.92	92.83	87.35	90.09	96.93	97.49	97.21	94.18	95.70	94.94	90.67	95.88	93.28

X

Table A7.2.1-4 Composition of Extractable Radioactivity – Manningtree soil (cont.)

Time	Day 41			Day 80			Day 149			Day 252			Day 336		
Replicate	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**
Compound Fipronil	75.03	71.19	73.11	58.16	51.98	55.07	30.04	32.44	31.24	28.96	21.83	25.40	12.12	20.26	16.19
XXXX	7.75	9.67	8.71	18.59	16.85	17.72	32.40	31.27	31.84	28.96	34.51	31.74	38.29	33.11	35.70
XXXX				0.59		~~0.59~~ **0.30**									
XXXX	2.53	2.71	2.62	2.50	3.05	2.78	4.44	3.96	4.20	3.12	4.76	3.94	3.67	4.88	4.28
XXXX	7.60	7.05	7.33	10.54	11.44	10.99	17.10	15.38	16.24	19.01	19.47	19.24	24.39	20.46	22.43
Component 1															
Component 2				1.65		~~1.65~~ **0.83**	1.75	1.79	1.77	1.67	2.23	1.95	3.00	3.32	3.16
Component 3														0.43	0.43
Component 4													1.04		1.04
Component 5										3.51		~~3.51~~ **1.75**			
Component 6											2.32	~~2.32~~ **1.16**			
Total recovery	92.91	90.62	91.77	92.03	83.32	87.68	85.73	84.84	85.29	85.23	85.12	85.18	82.51	82.46	82.49

Overall mean recovery : 91.33%

Table A7.2.1-5 Composition of Extractable Radioactivity – Speyer 2.2 soil

Time	Day 0			Day 1			Day 3			Day 7			Day 14			Day 30		
Replicate	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**
Compound																		
Fipronil	101.71	101.81	101.76	101.62	100.31	100.97	93.94	95.96	94.95	92.78	96.18	94.48	88.05	89.46	88.76	72.10	77.23	74.67
XXXX								0.73	~~0.73~~ **0.37**	1.36	1.52	1.44	3.21	2.21	2.71	9.15	9.10	9.13
XXXX																		
XXXX													0.90		~~0.90~~ **0.45**			
XXXX										0.88	0.49	0.69	1.64	1.22	1.43	2.27	1.34	1.81
XXXX							0.76	1.19	0.98	3.23	2.33	2.78	2.42	4.16	3.29	6.80	6.15	6.48
Component 1													0.55		~~0.55~~ **0.28**			
Component 2														0.91	~~0.91~~ **0.45**			
Component 4																		
Total recovery	101.71	101.81	101.76	101.62	100.31	100.97	94.7	97.88	96.29	98.25	100.52	99.39	96.77	97.96	97.37	90.32	93.82	92.07

X

Time	Day 41			Day 80			Day 149			Day 252			Day 336		
Replicate	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**	**A**	**B**	**Mean**
Compound															
Fipronil	71.76	75.67	73.72	65.16	68.45	68.81	60.41	59.24	59.83	52.11	55.57	53.84	45.57	44.23	44.90
XXXX	12.56	11.92	12.24	13.27	14.47	13.87	15.04	20.21	17.63	24.54	26.65	25.60	26.00	26.71	26.36
XXXX										0.76	0.57	**0.67**		0.74	~~0.74~~ **0.37**
XXXX				0.62	0.29	0.46									
XXXX	1.84	2.37	2.11	1.26	1.31	1.29	1.57	1.74	1.66	2.90	3.16	3.03	3.13	1.71	2.42
XXXX	7.50	6.97	7.24	9.30	7.75	8.53	9.55	9.29	9.42	11.54	10.54	11.04	13.23	14.30	13.77
Component 1															
Component 2				0.94	0.99	0.97	0.70	1.33	1.02	1.38	1.74	1.56	2.20	2.94	2.57
Component 4													3.12	1.10	2.11
Total recovery	93.66	96.93	95.30	90.55	93.26	91.91	87.27	91.81	89.54	93.23	98.23	95.73	93.25	91.73	92.49

Overall mean recovery : 95.71%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Applicant's version is acceptable with the following comment: **3.2.3 Study design** Incubation units were treated with 0.44 µCi in up to 525µl of acetonitrile, not 0.45 µCi in up to 0.5 ml as stated in the protocol. A temperature range of 21-28°C was recorded during the test, thus the temperature has not been maintained constant to 20±2°C as recommended by the OCDE Guideline 307 or 25±1°C as recommended in the USEPA Guidelines. However, these deviations are not considered as having affected the outcome of the study in a significant way and they are minor deviations. Considering the complement of this study "Thermograph records to address comments from RMS on the incubation temperature in the study of Waring (1993) XXXX", we consider that the test was done at 25°C for the recalculation of the half-lives to reflect EU outdoor temperature of 12°C in document II. Those recalculations indicates half-lives of 362 and 871 days respectively.
Conclusion	Agree with the applicant's version with the following revision : *The half life of [^{14}C]-Fipronil determined by HPLC in Manningtree and Speyer 2.2 soils under aerobic conditions were 128 and 308 days respectively, degradation proceeded via hydrolysis to XXXX and oxidation to XXXX.* The recalculation of the half-lives to reflect EU outdoor temperature od 12°C indicates half-lives of 362 and 871 days respectively.
Reliability	1
Acceptability	Acceptable
Remarks	Errors in Table A7.2.1-2, A7.2.1-3, A7.2.1-4, A7.2.1-5 were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.2 **Annex Point IIIA, XII.1.1**	**Aerobic degradation in soil, further studies**

Section 7.2.2.1 **Annex Point IIIA, VII.4, XII.1.1, XII.1.4**	**Aerobic degradation in soil – further study**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.2.2.1/01** Fitzmaurice, M. J. and Mackenzie, E. [^{14}C]-Fipronil: Degradation in Four Soils at 20°C and Two Soils at 10°C January 2002 (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	Yes	
2.3	**Deviations**	The study protocol states that extracts generated during the study would be profiled within 28 days of production. This time period was exceeded in two cases. Since storage stability data were obtained to show that extracts were stable for more than 120 days under the storage conditions used, this deviation is not considered to have affected the integrity of the study	
		3. MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C]-Fipronil	
3.1.1	Lot/Batch number	PJS107/1	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	Radiopurity 99.25% (HPLC); 96% (TLC)	
3.1.4	Further relevant properties	Specific radioactivity 2.77MBq/mg	
3.1.5	Composition of product	Not applicable	

Section 7.2.2.1 **Annex Point IIIA, VII.4, XII.1.1, XII.1.4**	**Aerobic degradation in soil – further study**	
3.2 Testing procedure		
3.2.1	This study was conducted according to the current EU guidelines for the rate of degradation of the active substance in three contrasting soil types. The four soils which covered a range of pH values, organic matter and sand/silt/clay contents were collected fresh from the field and handled and stored according to the ISO standard (ISO/DIS 10381-6) to maintain microbial viability. The soil characteristics are given in Table A7.2.2.1-1. Portions (100g dry soil equivalent) of each soil were weighed into individual incubation flasks and the soil moisture content adjusted to 45% of maximum water holding capacity. The soil samples were treated with a nominal 0.02 mg of [^{14}C]-Fipronil in 230µl of acetonitrile and the solvent allowed to evaporate. This was equivalent to 184 g/ha. The soils were then incubated at 20°C and 10°C for up to 219 days. Volatile products were collected in a series of trapping solutions. The samples were extracted with acetonitrile and acetonitrile/water and/or acetone by shaking at room temperature or by soxhlet extraction with acetonitrile/water. The soil samples were then air-dried, ground to a fine powder and the residual radioactivity present quantified. Solvent extracts were concentrated and analysed by reverse phase HLC using known certified reference standards. Structural confirmation of the identity was obtained by LC-MS/MS.	
	4. RESULTS	
4.1	Tables A7.2.2.1-2 to 14	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The aerobic degradation of [^{14}C]-Fipronil in four soils (at 20°C) and two soils (at 10°C) was investigated according to Current EU Guidelines	
5.2 Results and discussion	A good radiochemical recovery was obtained with the mean total recovery value for all soil types being 100.9% and individual recoveries being between 95 and 107%.	
	Fipronil ws steadily degraded by hydrolysis to XXXX and oxidation to XXXX. The rate of degradation was dependent upon temperature and soil microbial biomass, being more rapid at 20°C than 10 °C and more rapid with higher microbial biomass. DT_{50} values ranged from 26 to 296 days at 20°C. The rate of degradation at 10°C was slower (DT_{50} values ranged from 364 to 686 days).	X
	The level of unextractable soil bound residues were low. Soil bound residues reached a maximum of ca. 10% after 28 days (clay loam (01/02) soil) and ca 7% after 3 days incubation (clay loam (01/01) soil) in soil incubated at 20°C and 10°C respectively. Virtually no volatile products were detected (<1% of applied radioactivity).	X

Section 7.2.2.1 **Annex Point IIIA, VII.4, XII.1.1, XII.1.4**	**Aerobic degradation in soil – further study**	
	Three major metabolites were detected in soils incubated at 20°C by hydrolysis XXXX, by oxidation XXXX and by reduction XXXX. XXXX and XXXX were detected in all four soils at levels exceeding 11% of applied radioactivity (maximum 38.9% and 34.4% of applied radioactivity, respectively). XXXX was detected at minor levels in three soils but exceeded 10% of applied activity in clay loam (01/02) soil (maximum 16.8%). This sol has a lower redox status than the other soils and this is the likely reason for the formation of the reduced metabolite XXXX due to reduced oxygen content, resulting in reduction of fipronil. Further degradation by hydrolysis of XXXX to XXXX and XXXX to XXXX was observed in the high pH and high microbial biomass soil in which XXXX reached a maximum of 8.5% and XXXX a maximum of 5.4%. Other unidentified metabolites did not exceed 7% of applied radioactivity. A proposed metabolic pathway is given in figure A7.2.2.1-1. Due to the poor fit criterion (correlation coefficient) to first order kinetics with Excel the KIM model is a superior fit to the data an attempt to derive DT50 values for the metabolites was made using TOPFIT and no reliable estimates could be obtained	
5.3 Conclusion	Fipronil was steadily degraded under aerobic conditions by hydrolysis to XXXX and by oxidation to XXXX. The rate of degradation was temperature dependent with more rapid degradation at 20°C than 10°C. The rate of degradation was also related to the soil microbial biomass activity. The reduced metabolite XXXX was found in minor quantities, except in one soil where there was reduced oxygen status under these laboratory conditions. Several other minor metabolites were also observed, the hydrolysis products RP 200761 and XXXX in the high pH and high biomass soil. The DT_{50} of fipronil ranged from 26 to 296 days at 20°C and the DT_{90} from 85 to 982 days. It was not possible to derive the DT values for the metabolites.	X
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A7.2.2.1-1 Soil Characteristics

Characteristic	Value			
Location	Chazay (FR)	Ongar (UK)	Royston (UK)	Levington (UK)
OS Map Reference	Not applicable	TL 557047	TL 365397	TM 098514
Soil Designation	00-30	01-01	01-02	01-03
Particle Size Analysis Coarse/Very Coarse Sand (500µm – 2mm) Medium Sand (250µm – 500µm) Fine Sand (106µm – 250µm) Very Fine Sand (63µm – 106µm) Coarse Silt (20µm – 63µm) Fine Silt (2µm – 20µm) Clay (<2µm)	% 8.12 9.44 13.55 10.44 9.51 15.58 33.38	% 7.96 7.06 12.19 6.22 14.47 19.96 29.14	% 5.35 2.99 4.66 7.04 14.51 31.03 34.40	% 14.59 30.02 24.38 4.37 6.37 9.43 10.83
Textural Class USDA and ADAS	Clay Loam	Clay Loam	Clay Loam	Sandy Loam
Organic Carbon	1.1	2.0	4.1	1.3
Organic Matter	1.9	3.4	7.1	2.2
Cation Exchange Capacity, mEq/100g	12.8	18.1	45.3	3.8
pH Deionised water 0.01M Calcium Chloride 1M Potassium Chloride	 8.2 7.3 7.6	 7.3 6.7 7.1	 8.3 7.1 8.2	 6.6 4.7 4.5
Water Holding Capacity, % w/w	45.32	60.11	104.67	39.27
Initial Microbial Biomass, µgC/g	176	359	1093	145
Final Microbial Biomass µg C/g 20°C	63	265	957	58
Final Microbial Biomass µg C/g 10°C	113	327		

Table A7.2.2.1-2 Distribution of Radioactivity in Clay Loam (00-30) Treated with [^{14}C]-Fipronil incubated at 20°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	101.85	0.85	102.70
3	0.04	96.09	5.38	101.51
7	0.04	98.54	3.68	102.26
14	0.04	95.20	6.17	101.41
28	0.06	98.01	4.10	102.17
56	0.05	96.57	3.93	100.55
91	0.07	92.85	6.31	99.23
120	0.08	92.00	3.87	95.94
153	0.10	96.37	3.81	100.29
182	0.12	100.12	2.13	102.36
219	0.09	96.16	3.40	99.65
Overall mean recovery				**100.73**

Table A7.2.2.1-3 Distribution of Radioactivity in Clay Loam (00-30) Treated with [^{14}C]-Fipronil incubated at 10°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	99.26	3.52	102.78
3	0.03	96.55	5.51	102.09
7	0.03	99.57	5.94	105.53
14	0.04	97.02	4.57	101.63
28	0.02	96.77	4.56	101.35
56	0.04	93.56	6.11	99.72
91	0.03	97.24	4.10	101.37
120	0.01	97.38	3.35	100.75
153	0.04	93.66	3.25	96.95
182	0.04	99.40	0.99	100.43
219	0.05	98.23	1.88	100.16
Overall mean recovery				**101.16**

Table A7.2.2.1-4 Distribution of Radioactivity in Clay Loam (01-01) Treated with [^{14}C]-Fipronil incubated at 20°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	98.59	3.14	101.73
3	0.04	94.59	6.83	101.46
7	0.03	99.28	3.95	103.26
14	0.03	97.59	8.64	106.26
28	0.03	98.21	8.22	106.46
56	0.04	94.39	4.11	98.54
91	0.07	91.80	5.93	97.80
120	0.08	92.10	6.84	99.02
153	0.15	92.52	5.23	97.90
182	0.11	98.93	2.74	101.78
219	0.12	97.02	4.07	101.21
Overall mean recovery				**101.45**

Table A7.2.2.1-5 Distribution of Radioactivity in Clay Loam (01-01) Treated with [^{14}C]-Fipronil incubated at 10°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	96.84	4.81	101.65
3	0.03	94.52	7.48	102.03
7	0.02	95.56	6.18	101.75
14	0.04	97.43	3.95	101.42
28	0.02	98.84	6.99	105.85
56	0.05	96.96	2.41	99.43
91	0.02	95.55	4.81	100.38
120	0.02	91.97	3.67	95.66
153	0.05	93.14	2.61	95.79
182	0.04	95.28	~~1.62~~ **1.82**	97.14
219	0.06	97.81	2.61	100.48
Overall mean recovery				**100.14**

X

Table A7.2.2.1-6 Distribution of Radioactivity in Clay Loam (01-02) Treated with [14C]-Fipronil incubated at 20°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	96.82	4.91	101.73
3	0.04	95.54	7.86	103.44
7	0.04	97.32	8.86	106.22
14	0.05	95.90	8.47	104.42
28	0.04	93.35	10.73	104.13
56	0.06	92.40	7.08	99.55
91	0.04	90.24	9.20	99.48
120	0.07	92.92	6.41	99.39
153	0.06	90.76	7.37	98.18
182	0.04	95.02	6.55	101.61
219	0.07	92.49	7.65	100.21
Overall mean recovery				**100.67**

Table A7.2.2.1-7 Distribution of Radioactivity in Clay Loam (01-03) Treated with [14C]-Fipronil incubated at 20°C

Time (Days	% Applied radioactivity			
	Volatiles	Extracted	Bound	Total
0	n.a	100.54	0.98	101.53
3	0.03	97.27	3.89	101.19
7	0.02	96.55	2.89	99.47
14	0.02	98.16	3.02	101.20
28	0.02	98.77	2.96	101.75
56	0.04	97.72	5.38	103.13
91	0.01	94.63	4.23	98.88
120	0.01	98.23	2.81	101.04
153	0.04	94.54	2.95	97.54
182	0.05	95.02	2.05	97.12
219	0.02	98.58	3.06	101.66
Overall mean recovery				**100.41**

Table A7.2.2.1-8 HPLC Analysis of Clay Loam (00-30) Extracts incubated at 20°C (Composition as % Applied Radioactivity)

Time (Days	RPA 105048	XXXX	XXXX	XXXX	XXXX	Fipronil	XXXX	XXXX
0						101.85		
3						96.09		
7						97.17	0.33	1.04
14						91.66	1.18	2.36
28				3.83		83.78	1.16	2.15
56				4.37		82.72		9.47
91				8.81		74.10	1.14	8.79
120				12.87		69.41	1.91	7.79
153				8.92		73.35	1.91	9.69
182				10.54		76.55	1.96	11.06
219				19.31		64.40	2.07	10.38

Table A7.2.2.1-9 HPLC Analysis of Clay Loam (00-30) Extracts incubated at 10°C (Composition as % Applied Radioactivity)

Time (Days	RPA 105048	XXXX	XXXX	XXXX	XXXX	Fipronil	XXXX	XXXX
0						99.26		
3						95.02		1.53
7					1.42	96.43		1.72
14						93.86	1.01	2.14
28						80.67	0.90	1.88
56				2.04		86.91		4.61
91				3.81		86.67	0.92	5.84
120				5.47		86.34	0.88	4.70
153				2.82		81.98		6.46
182				2.43		89.61	1.48	5.98
219				5.12		87.48		5.63

Table A7.2.2.1-10 HPLC Analysis of Clay Loam (01-01) Extracts incubated at 20°C (Composition as % Applied Radioactivity)

Time (Days	RPA 105048	XXXX	XXXX	XXXX	XXXX	Fipronil	XXXX	XXXX
0						98.59		
3						94.05	0.54	
7						94.61	0.59	4.08
14				3.31		84.52	3.88	5.88
28				9.03		72.69	4.05	6.89
56				11.73		64.89	6.79	10.98
91				17.72		50.66	7.66	15.76
120				23.00		42.31	7.79	19.00
153				21.33		35.28	7.20	22.43
182				30.04		40.59	8.63	19.67
219				38.44		29.30	8.22	21.05

Table A7.2.2.1-11 HPLC Analysis of Clay Loam (01-01) Extracts incubated at 10°C (Composition as % Applied Radioactivity)

Time (Days	RPA 105048	XXXX	XXXX	XXXX	XXXX	Fipronil	XXXX	XXXX
0						96.84		
3				2.63		88.00	1.24	2.65
7						94.35	0.34	0.86
14						93.68	1.42	2.33
28						88.17	2.04	2.63
56				3.05		82.31	3.31	8.30
91				4.77		81.27	2.04	7.48
120				9.28		70.20	1.32	11.16
153				6.35		77.29		6.72
182				4.65		84.21		6.41
219				13.22		66.64	3.36	14.59

Table A7.2.2.1-12 HPLC Analysis of Clay Loam (01-02) Extracts incubated at 20°C (Composition as % Applied Radioactivity)

Time (Days	Peak A	Unident-ified B	XXXX	Unident-ified C	XXXX	XXXX	Fipronil	XXXX	XXXX
0							96.82		
3							94.76		0.79
7	2.76					3.43	82.70	2.53	5.91
14	1.68					8.81	68.85	6.78	9.77
28						12.53	56.38	7.99	16.45
56		1.89	1.95		2.23	~~19.76~~ **21.73**	25.27	15.33	24.00
91		2.30	2.32		2.76	~~18.32~~ **22.70**	14.37	16.99	28.82
120		2.22	4.67		3.34	23.92	8.63	16.78	29.80
153		1.70	3.02	1.00	4.04	20.48	6.16	14.64	30.35
182		2.93	6.67	1.76	4.79	25.17	5.58	13.78	34.34
219		6.48	8.46	2.84	5.40	22.72	3.53	12.31	30.76

X

Table A7.2.2.1-13 HPLC Analysis of Clay Loam (01-03) Extracts incubated at 20°C (Composition as % Applied Radioactivity)

Time (Days	RPA 105048	XXXX	XXXX	XXXX	XXXX	Fipronil	XXXX	XXXX
0						100.54		
3						96.08		1.19
7				1.08		95.03	0.44	
14				2.30		93.92	0.56	1.38
28				9.90		78.43	3.99	3.17
56				15.54		72.54	3.31	6.34
91				19.28		64.26	3.49	7.60
120				19.62		69.02	2.07	7.53
153				15.70		57.48		13.25
182				15.17		71.31	1.09	7.46
219				29.01		57.94	2.08	9.54

Table A7.2.2.1-14a Kinetic Analysis for the Degradation of Fipronil under Aerobic Conditions : KIM Model

Soil Type	DT_{50} Days313	DT_{90} Days	Fit Criterion b value	Best-Fit Model
Clay Loam (00-30) at 20°C	304	1010	-0.996	1st order
Clay Loam (00-30) at 10°C	686	2279	-0.994	1st order
Clay Loam (01-01) at 20°C	102	339	-0.995	1st order
Clay Loam (01-01) at 10°C	358	1189	-0.994	1st order
Clay Loam (01-02) at 20°C	31	102	-0.997	1st order
Clay Loam (01-03) at 20°C	221	734	-0.991	1st order

Table A7.2.2.1-14b Kinetic Analysis for the Degradation of Fipronil under Aerobic Conditions : First Order (Excel Model)

Soil Type	DT_{50} Days313	DT_{90} Days	Fit Criterion r^2
Clay Loam (00-30) at 20°C	313	1041	0.77
Clay Loam (00-30) at 10°C	700	2326	-0.43
Clay Loam (01-01) at 20°C	104	346	0.95
Clay Loam (01-01) at 10°C	370	1230	0.57
Clay Loam (01-02) at 20°C	39	131	0.96
Clay Loam (01-03) at 20°C	214	710	0.80

Figure A7.2.2.1-1 Proposed Metabolic Pathway of Fipronil in Soil

XXXX

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with the applicant's version. The study protocol states that extracts generated during the study would be profiled within 28 days of production. This time period was exceeded in two cases. Since storage stability data were obtained to show that extracts were stable for more than 120 days under the storage conditions used, this deviation is not considered to have affected the integrity of the study
Conclusion	Agree with the applicant's version with the following revision : **5.2 Results and discussion** *DT_{50} values ranged from ~~26 to 296~~ 31 to 304 days at 20°C. The rate of degradation at 10°C was slower (DT_{50} values ranged from ~~364 to 686~~ 358 to 686 days). The level of unextractable soil bound residues were low. Soil bound residues reached a maximum of ca. ~~10%~~ 11% after 28 days (clay loam (01/02) soil) and ca 7% after 3 days incubation (clay loam (01/01) soil) in soil incubated at 20°C and 10°C respectively.* 5.3 Conclusion *The DT_{50} of fipronil ranged from ~~26 to 296~~ 31 to 304 days at 20°C and the DT_{90}* from ~~85 to 982~~ 102 to 1010 days. At 10°C, the DT_{50} of fipronil ranged from 358 to 686 days, and the DT_{90} from 1189 to 2279 days. It was *not possible to derive the DT values for the metabolites.*
Reliability	1
Acceptability	Acceptable
Remarks	Errors in Table A7.2.2.1-5, A7.2.2.1-12, were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.2.2.2/01** Wicks, R. (2005) Fipronil: Long term soil dissipation study in Northern Europe with repeated applications (final data after 6 years) (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 95/36; EEC 91/414	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	EXP 60720A	
3.1.1	Lot/Batch number	Not recorded	
3.1.2	Specification	Not recorded	
3.1.3	Purity	800 g/kg Experimental wettable powder	
3.1.4	Further relevant properties	None recorded	
3.1.5	Method of analysis	GC with electron capture Year 4 onwards GC/MS	
3.2	**Degradation products**		
3.2.1	Method of analysis for degradation products	As for the active substance	
3.3	**Reference substance**	None	
3.4	**Soil types**	See Table 7.2.2.2.2-1	
3.5	**Testing procedure**	The environmental behaviour of fipronil and its metabolites was studied following soil incorporation at two location in Northern Europe.	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**	
	In the autumn of each year starting 1999 in France and in spring starting 2000 in Belgium, fipronil was sprayed at a nominal rate of 200 g a.s./ha onto the soil surface and incorporated prior to planting seed. Wheat was grown each year in France while in Belgium maize and sugar beet were rotated. Soil samples from depths of 0 to 20 cm and 20 to 30 cm below the surface were then collected just after application, 6 to 9 months later and just before the next application. Soil samples were analyzed for fipronil and its metabolites, XXXX, XXXX, XXXX and XXXX. Trial plots were ploughed to a depth of about 25 to 30 cm and then cultivated before application according to normal agricultural practice. After incorporation of the test substance by rotary harrow, crop was planted according to normal practice (wheat at Arras and maize/sugar beet alternating at Kortenaken). The plot at Arras had a very minor slope of 1%. The treated plot was 20 x 80 m divided into four equal subplots. Rainfall usually exceeded the monthly historical rainfall and during the period of the study. The soil characteristics of the field site Arras in Northern Europe are given in Table A7.2.2.2-1. The plot at Kortenaken was not drained and had no slope. The treated plot was 20 x 80 m divided into four equal subplots. Rainfall usually exceeded the monthly historical rainfall and during the period of the study. The soil characteristics of the field site Kortenaken in Northern Europe are given in Table A7.2.2.2-1. Soil samples were taken prior to treatment and several sampling dates thereafter. After application, twenty soil cores were taken, five in each of the four subplots from different depth down to 30 cm (0-20, 20-30 cm). The LOQ was 0.002 mg kg^{-1} (wet weight).	
	4. RESULTS	
4.1	The fipronil results from filter papers were within the acceptable recovery range of 70 to 110%. Fipronil residues in the immediate post-application soil samples were consistent with the calibrated application rate at both trial sites. The variability in soil residues is probably due in part to differing soil conditions at each application and to the incorporation of fipronil resulting in variability in soil density.	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**	
	Fipronil residues in the immediate post-application soil samples at the last application were approximately 180% and 280% of the calibrated application rate at the two trials. The fipronil formulation applied was the same specification as used in previous years and the filter paper results were within the range of previous years indicating that the correct application rate was used. The procedures used for incorporation and soil samplings were the same as in previous years. Analysis of a second replicate set of soil samples using new stock and working standards produced similar results. No evidence of contamination of soil samples was found because the control samples were free of residues. There is currently no obvious explanation for the high residues in the soil samples taken immediately after the last application and further investigations are being undertaken. Soil residues of fipronil and metabolites XXXX, XXXX, XXXX and XXXX expressed in µg/kg and in g/ha are given in Table A7.2.2.2-2 and Table A7.2.2.2-3 for the site "Arras" and in Tables A7.2.2.2-4 and A7.2.2.2-5 for the site "Kortenaken", respectively.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The environmental behaviour of fipronil and its metabolites was studied following The soil incorporation of an experimental wettable powder at two location in Northern Europe.	
5.2 Results and discussion	The trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to less than half in mid application samples (6 to 9 months after application) and below or close to the limit of quantification (LOQ), when averaged over the soil profile to 30 cm, one year after application at the two sites.	
	The major metabolite found was XXXX with significant amounts of XXXX. XXXX residues generally peaked at the mid-way sampling point between applications but residues were still present one year after each application. The amounts of XXXX residue were less than for XXXX but the trend was similar. The pattern of residues for XXXX was similar to XXXX but the amounts of XXXX found were only about 10 to 20% of XXXX. Residues of the photolyte, XXXX, remained below LOQ for both trials, which is consistent with soil incorporation of the parent compound.	X
	The residues of the metabolites XXXX, XXXX and XXXX which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.	
5.3 Conclusion		
5.3.1 Reliability	1	X
5.3.2 Deficiencies	None	

Table A7.2.2.2-1Soil characteristics of the fipronil field sites in Northern Europe (ADAS classification)

Site	Soil texture	Sand [%]	Silt [%]	Clay [%]	pH water [-]	OC [%]
Arras (France)	Silty clay loam	17	60	23	8.2	1.5
Kortenaken (Belgium)	Silt loam	14	72	14	6.8	1.2

Table Table A7.2.2.2-2 Soil residues at Arras expressed in µg kg^{-1} (dry weight)

Year	Days after first application	Sample increment [cm]	Fipronil [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]
1	0	0-20	82.5	NA	NA	NA	NA
		20-30	NA	NA	NA	NA	NA
	174	0-20	24.4	16.3	4.3	<LOQ	15.8
		20-30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	351	0-20	<LOQ	18.4	3.7	<LOQ	14.4
		20-30	<LOQ	33.0	4.8	<LOQ	7.9
2	351	0-20	69.2	22.0	4.1	<LOQ	11.8
		20-30	3.8	11.7	<LOQ	<LOQ	4.9
	608	0-20	20.8	57.8	10.1	<LOQ	17.7
		20-30	<LOQ	22.9	3.1	<LOQ	9.6
	734	0-20	2.6	31.3	5.1	<LOQ	13.7
		20-30	<LOQ	24.1	4.7	<LOQ	9.4
3	734	0-20	82.4	58.2	9.3	<LOQ	16.0
		20-30	6.0	22.5	4.1	<LOQ	8.2
	944	0-20	30.5	82.9	15.9	<LOQ	33.2
		20-30	3.8	38.8	6.5	<LOQ	17.3
	1085	0-20	2.3	56.9	9.4	<LOQ	20.0
		20-30	<LOQ	43.6	7.3	<LOQ	16.4
4	1085	0-20	75.5	56.8	9.5	<LOQ	24.4
		20-30	<LOQ	32.5	5.0	<LOQ	13.3
	1288	0-20	19.6	56.4	7.7	<LOQ	21.1
		20-30	3.3	44.5	6.6	<LOQ	16.5
	1463	0-20	3.0	56.3	8.8	<LOQ*	16.3
		20-30	<LOQ*	36.8	5.8	<LOQ*	13.3
5	1463	0-20	82.3	54.5	9.3	<LOQ*	17.3
		20-30	4.0	44.0	7.0	<LOQ*	13.5
	1694	0-20	26.0	95.3	13.3	<LOQ*	29.3
		20-30	<LOQ*	39.3	5.3	<LOQ*	15.0
	1833	0-20	3.3	65.5	9.5	<LOQ*	20.5
		20-30	<LOQ*	53.3	7.5	<LOQ*	15.0
6	1833	0-20	184.8	78.0	10.5	<LOQ*	26.4
		20-30	9.8	50.5	7.2	<LOQ*	19.4

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 2 µg kg^{-1} (wet weight)

<LOQ* = mean residue value was below the limit of quantification of 2 µg kg^{-1} (dry weight)

Table Table A7.2.2.2-3 Soil residues at Arras expressed in g ha^{-1}

Year	Days after first application	Fipronil (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})
1	0	247.4	NA	NA	NA	NA
	174	75.0	50.6	14.6	<LOQ	49.2
	351	<LOQ	104.6	18.3	<LOQ	54.9
2	351	213.4	83.4	15.5	<LOQ	42.7
	608	65.6	207.7	35.0	<LOQ	67.5
	734	10.9	129.9	22.3	<LOQ	55.2
3	734	256.3	208.1	34.0	<LOQ	60.3
	944	97.2	307.0	57.3	<LOQ	125.7
	1085	<LOQ	236.3	39.2	<LOQ	84.5
4	1085	228.8	219.2	36.1	<LOQ	93.1
	1288	63.8	235.8	32.9	<LOQ	88.2
	1463	10.5	223.9	34.9	<LOQ*	68.6
5	1463	252.8	229.5	38.3	<LOQ*	72.0
	1694	79.5	344.6	47.6	<LOQ*	110.3
	1833	12.0	276.4	39.8	<LOQ*	84.0
6	1833	569.3	309.8	42.3	<LOQ*	108.4

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 10.8 g ha^{-1} (dry weight basis assuming a soil wet to dry weight ratio of 1.2). <LOQ* = mean residue value was below 9 g ha^{-1}

Table Table A7.2.2.2-4 Soil residues at Kortenaken expressed in µg kg-1 (dry weight)

Year	Days after first application	Sample increment [cm]	Fipronil [µg kg^{-1}]	XXXX (µg kg^{-1})	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]
1	0	0-20	54.5	NA	NA	NA	NA
		20-30	NA	NA	NA	NA	NA
	188	0-20	23.9	28.7	4.5	<LOQ	15.3
		20-30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	330	0-20	3.2	7.0	<LOQ	<LOQ	13.7
		20-30	5.3	12.0	<LOQ	<LOQ	9.3
2	358	0-20	55.8	15.9	2.5	<LOQ	9.0
		20-30	13.0	28.8	4.7	<LOQ	8.5
	553	0-20	10.6	48.8	6.9	<LOQ	23.8
		20-30	3.1	16.1	3.4	<LOQ	8.1
	714	0-20	9.9	54.4	9.0	<LOQ	23.5
		20-30	3.7	20.9	3.1	<LOQ	13.7
3	728	0-20	76.7	44.3	8.4	<LOQ	20.6
		20-30	3.8	16.6	3.1	<LOQ	11.0
	925	0-20	15.1	72.4	15.2	<LOQ	39.3
		20-30	2.5	35.0	5.9	<LOQ	19.4
	1056	0-20	5.2	41.5	7.9	<LOQ	20.0
		20-30	8.7	58.4	12.5	<LOQ	23.4
4	1064	0-20	65.4	46.8	8.8	<LOQ	21.3
		20-30	6.9	41.8	7.8	<LOQ	17.5
	1233	0-20	32.3	48.0	8.8	<LOQ*	24.3
		20-30	4.8	59.8	12.5	<LOQ*	17.5
	1451	0-20	5.5	45.8	8.8	<LOQ*	18.3
		20-30	10.5	55.5	10.0	<LOQ*	18.5
5	1463	0-20	113.8	60.0	11.3	<LOQ*	24.0
		20-30	8.8	48.5	8.5	<LOQ*	18.9
	1650	0-20	22.8	56.3	9.5	<LOQ*	30.5
		20-30	9.5	47.0	8.8	<LOQ*	27.5

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 2 µg kg^{-1} (wet weight)

<LOQ* = mean residue value was below the limit of quantification of 2 µg kg^{-1} (dry weight)

Table Table A7.2.2.2-5 Soil residues at Kortenaken expressed in g ha^{-1}

Year	Days after first application	Fipronil [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]
1	0	163.6	NA	NA	NA	NA
	188	74.5	88.8	15.3	<LOQ	48.2
	330	17.5	39.0	<LOQ	<LOQ	55.0
2	358	186.9	90.9	14.6	<LOQ	39.9
	553	36.5	170.5	25.7	<LOQ	83.4
	714	35.3	194.4	31.5	<LOQ	91.0
3	728	235.7	157.7	30.0	<LOQ	78.2
	925	49.0	269.6	54.5	<LOQ	146.9
	1056	28.6	212.1	42.4	<LOQ	95.1
4	1064	206.4	203.2	38.2	<LOQ	90.1
	1233	103.9	233.6	45.0	<LOQ*	99.0
	1451	32.3	220.5	41.3	<LOQ*	82.5
5	1463	354.4	252.8	46.5	<LOQ*	100.3
	1650	82.5	239.3	41.6	<LOQ*	132.8

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 10.8 g ha^{-1} (dry weight basis assuming a soil wet to dry weight ratio of 1.2). <LOQ* = mean residue value was below 9 g ha^{-1}

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with the applicant's version.
Conclusion	Agree with the applicant's version.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.2.2.2/02** Wicks, R. 2005 Fipronil: Long term soil dissipation study in Southern Europe with repeated applications (final data after 6 years) (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 95/36; EEC 91/414	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	EXP 60720A	
3.1.1	Lot/Batch number	Bologna Yr 1 & 2 OP990218 Yr 3 & 4 OP990218 Yr 5 OP210663 Yr 6 OP230101 Saulce Yr 1 & 2 OP990918 Yr 3 OP200069 Yr 4 OP210663 Yr 5 OP230101	
3.1.2	Specification	Not recorded	
3.1.3	Purity	A nominal 800 g/kg Experimental wettable powder Actual concentrations OP990218 805 g/kg OP200069 797 g/kg OP210663 792 g/kg OP230101 807 g/kg OP990918 788 g/kg	
3.1.4	Further relevant properties	None recorded	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**	
3.1.5 Method of analysis	GC with electron capture Year 4 onwards GC/MS	
3.2 Degradation products		
3.2.1 Method of analysis for degradation products	As for the active substance	
3.3 Reference substance	None	
3.4 Soil types	See Table A7.2.2.2-6	
3.5 Testing procedure	The environmental behaviour of fipronil and its metabolites was studied following soil incorporation at two locations in Southern Europe. The field study was carried out at two locations in Southern Europe: Bologna, Italy and Saulce sur Rhône, France. In the spring of each year starting 1999 in Italy and 2000 in France, fipronil was sprayed at a nominal rate of 200 g a.i. ha^{-1} onto the soil surface and incorporated prior to planting maize seed. Soil samples from depths of 0 to 20 cm and 20 to 30 cm below the surface were then collected just after application, after harvest of crop and just before the next application. Soil samples were analyzed for fipronil and its metabolites, XXXX, XXXX, XXXX and XXXX. Trial plots were ploughed to a depth of about 25 to 30 cm and then cultivated generally by harrow before application according to normal agricultural practice. After incorporation of the test substance by cultivator or rotary harrow, maize seed was planted according to normal practice. The plot at Bologna was not drained and had no slope. The treated plot was 32 x 40 m divided into four equal subplots (16 x 20 m). Rainfall usually exceeded the monthly historical rainfall and during the period of the study. The soil characteristics of the field site Bologna in Southern Europe are given in Table A2.2.2.-6. The plot at Saulce sur Rhône had no slope. The treated plot was 73 x 15 m divided into four equal subplots (17 x 15 m). Rainfall usually exceeded the monthly historical rainfall and during the period of the study. The soil characteristics of the field site Saulce sur Rhône in Southern Europe are given in Table A2.2.2-6.	
	4. RESULTS	
4.1	The fipronil results from filter papers were within the acceptable recovery range of 70 to 110 % or in several cases slightly below this range. Fipronil residues in the immediate post-application soil samples were consistent with the calibrated application rate at both trial sites. The variability in soil residues is probably due in part to differing soil conditions at each application and to the incorporation of fipronil resulting in variability in soil density.	

Section 7.2.2.2 **Annex Point IIIA, XII.1.1**	**Field soil dissipation and accumulation**	
	Fipronil residues in the immediate post-application soil samples at the last application were approximately 175 % of the calibrated application rate at both trials. The fipronil formulation applied was the same specifications as used in previous years and the filter paper results were within the range of previous years indicating that the correct application rate was used. The procedures used for incorporation and soil sampling were the same as in previous years. Analysis of a second replicate set of soil samples using new stock and working standards produced similar results. No evidence of contamination of soil samples was found because the control samples were free of residues. There is currently no obvious explanation for the high residues in the soil samples taken immediately after the last application and further investigations are being undertaken. Soil residues of fipronil and metabolites XXXX, XXXX, XXXX and XXXX expressed in µg/kg and in g/ha are given in Table A2.2.2-7 and Table A2.2.2-8 for the site Bologna and in Table A2.2.2-9 and Table A2.2.2-10 for the site of Saulce, respectively.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The environmental behaviour of fipronil and its metabolites was studied following The soil incorporation of an experimental wettable powder at two location in Southern Europe.	
5.2 Results and discussion	Based on the analytical results available up to the fifth and sixth years for the trials in France and Italy respectively, the trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to below LOQ by one year after application at both trial sites. No residues of the photolyte, XXXX, were found (apart from 2 detects) which is consistent with soil incorporation of the parent compound. The residues of the metabolites XXXX, XXXX and XXXX which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.	X
5.3 Conclusion		
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A2.2.2-6 Soil characteristics of the fipronil field sites in Southern Europe (ADAS classification)

Site	Soil texture	Sand [%]	Silt [%]	Clay [%]	pH water [-]	OC [%]
Bologna (Italy)	Clay loam	42	33	25	8.6	0.9
Saulce sur Rhône (France)	Sandy silt loam	33	50	17	8.0	0.9

Table A2.2.2-7 Soil residues of fipronil and metabolites XXXX, XXXX, XXXX and XXXX expressed in µg/kg (dry weight); Bologna

Year	Days after first application	Sample increment [cm]	Fipronil [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]
	0	0-20	38.4	NA	NA	NA	NA
		20-30	<LOQ	NA	NA	NA	NA
	127	0-20	2.9	13.3	2.6	<LOQ	11.7
		20-30	<LOQ	<LOQ	<LOQ	<LOQ	2.3
	365	0-20	<LOQ	5.0	<LOQ	<LOQ	4.7
		20-30	2.9	8.0	2.5	<LOQ	2.7
2	366	0-20	22.6	8.3	<LOQ	<LOQ	4.6
		20-30	6.9	3.5	<LOQ	<LOQ	2.6
	499	0-20	4.4	15.2	2.6	<LOQ	16.6
		20-30	<LOQ	4.0	<LOQ	<LOQ	4.3
	727	0-20	<LOQ	12.8	<LOQ	<LOQ	6.5
		20-30	<LOQ	10.1	<LOQ	<LOQ	6.8
3	728	0-20	51.1	29.0	5.2	<LOQ	9.5
		20-30	3.5	13.5	<LOQ	<LOQ	10.1
	881	0-20	4.1	41.8	6.2	<LOQ	17.6
		20-30	<LOQ	8.6	<LOQ	<LOQ	4.8
	1093	0-20	<LOQ	17.1	2.5	<LOQ	12.7
		20-30	<LOQ	22.6	2.8	<LOQ	5.8
4	1100	0-20	25.9	28.3	3.3	2.4	9.6
		20-30	9.5	14.1	<LOQ	<LOQ	10.1
	1245	0-20	5.8	36.9	6.1	<LOQ	20.4
		20-30	<LOQ	26.3	4.6	<LOQ	12.2
	1456	0-20	<LOQ	14.4	3.0	<LOQ	7.5
		20-30	<LOQ	15.6	2.8	<LOQ	9.3
5	1457	0-20	19.7	16.3	3.2	<LOQ	7.7
		20-30	2.5	20.6	3.1	<LOQ	8.1
	1598	0-20	10.3	42.3	7.8	<LOQ*	23.5
		20-30	<LOQ*	23.5	3.8	<LOQ*	11.5
	1826	0-20	<LOQ*	18.3	3.3	<LOQ*	5.5
		20-30	2.0	25.5	3.5	<LOQ*	10.5
6	1826	0-20	112.0	16.3	3.6	<LOQ*	5.5
		20-30	8.5	19.8	4.5	<LOQ*	9.5
	1974	0-20	7.8	32.8	5.0	<LOQ*	16.0
		20-30	<LOQ*	26.0	4.0	<LOQ*	10.8

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 2 µg kg^{-1} (wet weight)

<LOQ* = mean residue value was below the limit of quantification of 2 µg kg^{-1} (dry weight)

Table A2.2.2-8 Soil residues at Bologna expressed in g ha^{-1}

Year	Days after first application	Fipronil (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})	XXXX (g ha^{-1})
1	0	117.1	NA	NA	NA	NA
	127	<LOQ	41.7	<LOQ	<LOQ	38.4
	365	<LOQ	27.0	<LOQ	<LOQ	18.2
2	366	78.3	30.2	<LOQ	<LOQ	17.7
	499	14.8	51.5	<LOQ	<LOQ	56.3
	727	<LOQ	53.5	<LOQ	<LOQ	29.9
3	728	158.3	107.4	17.9	<LOQ	43.5
	881	14.1	138.2	20.3	<LOQ	60.1
	1093	<LOQ	85.1	11.6	<LOQ	46.9
4	1100	91.9	106.1	11.8	<LOQ	44.1
	1245	19.2	150.2	25.2	<LOQ	79.7
	1456	<LOQ	66.6	13.2	<LOQ	36.5
5	1457	62.9	79.8	14.4	<LOQ	35.3
	1598	33.0	162.0	28.9	<LOQ*	87.8
	1826	<LOQ*	93.0	15.0	<LOQ*	32.3
6	1826	348.8	78.4	17.6	<LOQ*	30.8
	1974	24.8	137.3	21.0	<LOQ*	64.1

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 10.8 g ha^{-1} (dry weight basis assuming a soil wet to dry weight ratio of 1.2). <LOQ* = mean residue value was below 9 g ha^{-1}

Table A2.2.2-9 Soil residues of fipronil and metabolites XXXX, XXXX, XXXX and XXXX expressed in µg/kg (dry weight); Saulce

Year	Days after first application	Sample increment [cm]	Fipronil [µg kg^{-1}]	XXXX (µg kg^{-1})	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]	XXXX [µg kg^{-1}]
1	0	0-20	63.6	NA	NA	NA	NA
		20-30	NA	NA	NA	NA	NA
	204	0-20	3.1	32.2	4.3	<LOQ	6.7
		20-30	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	359	0-20	2.4	17.8	3.3	<LOQ	6.5
		20-30	<LOQ	4.6	<LOQ	<LOQ	4.3
2	359	0-20	62.0	16.1	<LOQ	<LOQ	7.5
		20-30	<LOQ	2.8	<LOQ	<LOQ	2.5
	566	0-20	3.3	44.9	3.6	<LOQ	12.2
		20-30	<LOQ	3.6	<LOQ	<LOQ	2.4
	754	0-20	<LOQ	33.6	4.5	<LOQ	12.9
		20-30	<LOQ	15.5	<LOQ	<LOQ	6.7
3	754	0-20	73.2	49.2	7.3	2.7	16.1
		20-30	<LOQ	6.0	<LOQ	<LOQ	6.3
	908	0-20	<LOQ	32.3	4.6	<LOQ	13.7
		20-30	<LOQ	4.8	<LOQ	<LOQ	4.3
	1120	0-20	<LOQ	30.6	5.2	<LOQ	12.8
		20-30	<LOQ	16.5	2.7	<LOQ	7.4
4	1121	0-20	60.0	37.6	6.0	<LOQ	13.2
		20-30	<LOQ	17.6	<LOQ	<LOQ	7.5
	1253	0-20	8.0	48.0	6.5	<LOQ*	15.5
		20-30	<LOQ*	14.8	<LOQ*	<LOQ*	6.0
	1469	0-20	<LOQ*	34.0	4.0	<LOQ*	9.33
		20-30	<LOQ*	30.8	3.5	<LOQ*	8.3
5	1477	0-20	114.6	41.1	5.9	<LOQ*	10.5
		20-30	4.0	36.3	4.5	<LOQ*	8.8
	1636	0-20	7.8	69.0	8.0	<LOQ*	19.3
		20-30	2.0	30.3	3.5	<LOQ*	9.8

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 2 µg kg^{-1} (wet weight)
<LOQ* = mean residue value was below the limit of quantification of 2 µg kg-1 (dry weight)

Table A2.2.2-10 Soil residues at Saulce expressed in g ha^{-1}

Year	Days after first application	Fipronil [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]	XXXX [g ha^{-1}]
1	0	190.9	NA	NA	NA	NA
	204	11.0	98.5	14.7	<LOQ	22.1
	359	<LOQ	60.3	11.6	<LOQ	26.0
2	359	188.0	52.5	<LOQ	<LOQ	26.1
	566	11.6	140.0	12.5	<LOQ	40.1
	754	<LOQ	124.2	16.3	<LOQ	48.8
3	754	221.4	156.6	24.1	<LOQ	57.8
	908	<LOQ	104.0	16.0	<LOQ	47.6
	1120	<LOQ	116.6	19.7	<LOQ	49.6
4	1121	181.7	139.2	20.8	<LOQ	50.8
	1253	25.5	166.1	21.8	<LOQ*	55.5
	1469	<LOQ*	148.1	17.3	<LOQ*	40.1
5	1477	349.9	177.8	24.4	<LOQ*	45.0
	1636	26.3	252.4	29.3	<LOQ*	72.4

NA = Not analysed

<LOQ = mean residue value was below the limit of quantification of 10.8 g ha^{-1} (dry weight basis assuming a soil wet to dry weight ratio of 1.2). <LOQ* = mean residue value was below 9 g ha^{-1}

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	Agree with the applicant's version.
Conclusion	Agree with the applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.2.3 **Annex Point IIIA, XII.1.4**	**Extent and nature of bound residues**	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	This information can be obtained from the study submitted in Section 7.2.1.	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	We disagree with the applicant's justification. According to the study submitted in section 7.2.1, the un-extractable soil residue reach a maximum of ca 15% in the Manningtree and 6% in the Speyer soils respectively. According to the *Guidance on Data Requirements for active substances and biocidal products*, (Final draft, version 4.3.2 October 2000), a study of the extent and nature of bound residues is required when bound residues may be formed which account for more than 10% of the active substance added. Therefore this study should be done. However, the intended use of this product involves a limited exposure, that is why the submission of this study is not necessary.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.2.4 **Annex Point IIIA, XII.1.1**	**Other soil degradation studies**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The behaviour of Fipronil in soil compartment is adequately described by existing laboratory and field studies. In addition it is very unlikely that soil compartment would be exposed to Fipronil when used according to the label recommendations (See Chapter 5) therefore no further soil degradation study is required.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.3 **Annex Point IIIA, XII.1.2-1.3**	**Adsorption and mobility in soil, further studies**	

Section 7.2.3.1 **Annex Point IIIA, XII.1.2**	**Adsorption and desorption in accordance with the new test guideline ec c18**	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [x]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Adsorption data for Fipronil are provided in section 7.1.3 Adsorption/Desorption test.	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant's justification
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.2.3.2 **Annex Point IIIA, XII.1.3**	**Mobility in the soil**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.2.3.2/01** Godward, P.J., Quarmby, D.L. and Austin, D.J. M&B 46030^{14}C Leaching study with five soils (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA guideline 163-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	IHR 1465	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Specific activity 19.62 mCi mmole^{-1} Radiochemical purity 96.4% by TLC HPLC 98.3%	
3.1.4	Further relevant properties	None	
3.1.5	Composition of product	Not applicable	
3.2	**Testing procedure**		
3.2.1	Study purpose	The leaching properties of [^{14}C]-Fipronil either freshly applied or after aerobic incubation for 35 days in five European soil types have been studied in the laboratory in accordance with EPA guideline 163-1 at a rate equivalent to approximately 200g as/ha.	X
3.2.2	Soils	The soils: Speyer 2.2 (Germany), sandy loam (UK), loam (UK), sandy-clay loam-1 (France), sandy-clay loam-2 (France) were air-dried to constant weight and sieved through a 2 mm screen prior to use and then packed into segmented glass tubes to yield a soil column of 36cm. The soil characteristics are given I Table 7.2.3..2-1	

Section 7.2.3.2 **Annex Point IIIA, XII.1.3**	**Mobility in the soil**	
3.2.3 Preparation of the samples	Radio labelled [^{14}C]-Fipronil was dissolved in acetonitrile to give a dosing solution with a nominal concentration of 0.157 mg/ml. The freshly-prepared dosing solution was used for both the parent and aged phases of the study	
3.2.4 Immediate leaching	An aliquot (250µl) of the dosing solution was then applied evenly to the top of the soil column and the soil surface covered by a glass-fibre disc. Aliquots (500µl) of the dose check solutions were assayed for radioactivity by LSR. Results indicated that the application rate of fipronil was equivalent to 197.7g as/ha	
3.2.5 Aged leaching	Samples of the sandy loam soil and the Speyer 2.2 soil were adjusted to 75% of their moisture holding capacity at 1/3 bar. Portions of these soil types (each equivalent to 20g oven dried soil) were weighed into Petri dishes and each dish was treated with 250µl of dosing solution. The treated soil samples were stored in the dark at 22°C (±2°C) under aerobic conditions. Moist air was continually drawn over the soil surface. The 20g oven dried equivalent of the incubated soil was placed at the top of the column. Incubated Speyer 2.2 soil was placed on the Speyer 2.2 columns and the UK sandy loam was placed on the sandy loam columns. The amount of previously-aged product on each column was equivalent to that used for parent product. After addition of the incubated soil, the soil surface was covered with a glass-fibre disc as before	
3.2.5 Column leaching	Columns were leached with 0.005M calcium chloride solution (997.5ml) by dripping onto the top of the column at a rate which did not exceed the infiltration capacity of the soil and equivalent to 50.8cm rainfall over a period of 2-7 days depending on soil type and infiltration capacity of the soil column. Leachate and soil segments were then sampled and analysed for fipronil and soil metabolites	
	4. RESULTS	
4.1	See Tables A7.2.3.2-1 to 4	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The leaching properties of [^{14}C]-Fipronil either freshly applied or after aerobic incubation for 35 days in five European soil types have been studied in the laboratory in accordance with EPA guideline 163-1 at a rate equivalent to approximately 200g as/ha.	
5.2 Results and discussion	Good recoveries of radioactivity were obtained from the immediately leached columns (87.4% – 102.5%, mean = 97.69%), from the incubated samples (96.2% – 102.81%, mean = 99.24%) and from the aged leaching columns (89.53% – 101.26%, mean = 96.14%).	

Section 7.2.3.2 **Annex Point IIIA, XII.1.3**	**Mobility in the soil**	
	Immediate leaching: The percentage of applied radioactivity found in the leachates was very low in all but one duplicate sample, ranging from 0.2% to 1.2%, depending on the permeability of each type of soil used. In one of two duplicate columns from the UK sandy loam experiment, 8% of the applied radioactivity was found in the leachate. Due to the low quantity of radioactivity in the leachate, radiochemical analysis was not possible. The remaining applied radioactivity (73 – 99%) was found principally in the top 6cm column layer for all soil types except the UK sandy loam soil which contained an average of 51 – 54% applied radioactivity in the top 6cm layers and 31 – 37% applied radioactivity in the 6 -12 cm layers of the duplicate columns. The coefficients of distribution (Kd) calculated from the leachate values and the column parameters were found to be: loam, 222; French sandy-clay-loam2, 67; French sandy-clay-loam 1, 128; Speyer 2.2, 253 and sandy-loam, 35. HPLC analysis of soil extractable residues showed that both the Speyer 2.2 and the sandy-loam soils contained predominantly fipronil. The remaining three soils all contained significant amount of fipronil, as well as trace quantities of XXXX, XXXX and XXXX were also found in some soils. The results show a low mobility for fipronil in the five soils. *Previously aged product*: The percentage of applied radioactivity found in the leachates was consistently low, ranging from <1% to 4%, depending on the permeability of the soils used, similar to the immediate leaching results. Due to the low quantity of radioactivity in the leachate, radiochemical analysis ws not possible. The remaining applied radioactivity (81 – 94%) was found principally in the top 6cm column layer for all soil types except the UK sandy loam which contained on average 56% of applied radioactivity in the top 6cm layer of the duplicate columns. The coefficients of distribution (Kd) calculated from the leachate values and the column parameters were found to be : loam, 124.4; French sandy-clay-loam 2, 67.9; French sandy-clay-loam 1, 12.2; Speyer 2.2, 185.7; and sandy-loam 13.5. Analysis of soil extractable residues showed fipronil as the major component, with minor amounts of XXXX, XXXX, XXXX being determined, however in greater quantities than in the non-aged column leaching experiment. Structural confirmation of the metabolites was obtained by MS. The results show low mobility of fipronil and metabolites in the five soils.	
5.3 Conclusion	The results show a low mobility of fipronil and metabolites in the five soils.	
5.3.1 Reliability	1	X
5.3.2 Deficiencies	None	

Table A7.2.3.2-1 Soil Characteristics

Location	**Speyer 2.2 (DE)**	**Manningtree (UK)**	**Manningtree (UK)**	**Chazay (FR)**	**Chazay (FR)**
Soil Designation	91/6	91/8	91/10	91/11	91/12
Clay (%)	8	12	25	24	34
Silt (%)	9	11	29	18	19
Sand (%)	83	77	46	58	47
USDA Classification	Loamy sand	Sandy loam	Loam	Sandy Clay loam	Sandy Clay loam
pH	6.3	6.1	6.9	6.2	6.3
Organic matter (%)	5.70	0.57	7.23	1.98	2.71
Water content of air-dried soils (%)	1.14	0.60	3.99	1.36	2.59
Cation Exchange Capacity mEq/100g	10.76	7.15	36.51	12.57	20.41
Moisture holding capacity @ 1/3 bar	10.7%	9.2%	31.3%	21.0%	23.6%
Bulk density (g/cm^3)	1.46	1.59	1.30	1.55	1.31
Biomass (µg C/g soil)	471	58	1420	268	700
Microbial Count per g dry weight Bacteria Actinomycetes Fungi	 2.1×10^6 1.4×10^6 16.4×10^3	 $<1 \times 10^5$ 2.8×10^6 1.0×10^2	 7.6×10^6 1.4×10^6 2.7×10^3	 9.0×10^6 1.1×10^6 $3.8 \times$ ~~10^3~~ **10^4**	 7.6×10^6 2.1×10^6 2.3×10^4

X

Table A7.2.3.2-2 Recovery of radioactivity after immediate leaching

Soil Column		% Applied Radioactivity							
		Leachate	Soil Section (cm)						Total
			0-6	6-12	12-18	18-24	24-30	30-36	
Sandy loam	A	7.74	50.88	31.44	6.82	0.52	1.12	0.97	99.50
	B	0.78	53.44	36.64	8.02	1.46	1.06	1.03	102.53
Loam	A	0.31	97.61	0.95	0.19	0.18	0.08	0.08	99.40
	B	0.33	94.75	1.66	0.48	0.36	0.33	0.26	98.17
Clay loam 1	A	0.17	73.02	8.88	2.43	1.15	0.75	0.99	87.39
	B	0.40	90.50	4.65	1.28	0.58	0.33	0.18	97.91
Clay loam 2	A	0.72	85.33	8.02	0.68	0.17	0.24	0.14	95.31
	B	1.24	89.94	4.19	0.72	0.25	0.15	0.03	96.52
Speyer 2.2	A	0.31	98.84	0.73	0.12	0.05	0.17	0.26	100.48
	B	0.24	98.10	1.09	0.15	0.03	0.09	0.00	99.70
Overall mean recovery									**97.**69

Table A7.2.3.2-3 Recovery of radioactivity from the incubated soil samples

Sampling time (days)	Soil Type	% Applied radioactivity		
		Solvent extracts	Soil Residue	Total
0	Speyer 2.2	97.48	3.48	100.96
	Sandy loam	95.90	1.40	97.30
2	Speyer 2.2	94.70	1.79	96.49
	Sandy loam	94.43	1.77	96.20
7	Speyer 2.2	95.83	2.08	97.91
	Sandy loam	96.10	1.76	97.86
14	Speyer 2.2	97.89	1.63	99.52
	Sandy loam	97.64	1.69	99.33
21	Speyer 2.2	98.19	2.03	100.22
	Sandy loam	97.79	2.51	100.33
35	Speyer 2.2	99.10	2.83	101.94
	Sandy loam	99.75	3.11	102.86
Overall mean recovery				**99.**24

Table A7.2.3.2-4 Recovery of radioactivity after aged leaching

Soil Column		% Applied Radioactivity							
		Leachate	Soil Section (cm)						Total
			0-8	8-14	14-20	20-26	26-32	32-38	
Sandy loam	A	4.06	60.38	24.64	4.75	1.10	1.28	0.75	96.96
	B	2.50	51.93	30.80	8.98	1.83	1.67	1.62	99.33
Loam	A	0.55	92.87	5.06	0.95	0.69	0.65	0.48	101.26
	B	0.64	88.33	5.77	0.79	0.67	0.69	0.57	97.45
Clay loam 1	A	2.83	81.25	4.22	0.67	0.39	0.05	0.12	89.53
	B	2.16	82.00	4.42	0.85	0.04	0.01	0.09	89.57
Clay loam 2	A	1.29	84.94	6.68	2.86	0.76	0.12	0.31	96.95
	B	0.70	83.68	6.38	2.15	0.63	0.22	0.20	93.97
Speyer 2.2	A	0.41	92.04	2.92	1.07	0.29	0.02	0.09	96.83
	B	0.29	93.85	4.19	0.82	0.31	0.06	0.01	99.52
Overall mean recovery									**96.14**

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	Agree with the applicant's version with the following comment: **3.2 Testing procedure** The leaching properties of [^{14}C]-Fipronil after aerobic incubation for 35 days in five European soil types has been studied. According to the OCDE Guideline 312, the period of incubation must be long enough to allow the formation of transformation products, therefore an incubation time equal to the half-life of the product is recommend, without exceeding 120 days. All the half-live determined previously exceed 120 days, therefore the incubation time should have been 120 days. This is not a major deviation because the three products of transformation are presents after the day 7, (see study 7.2.1 and 7.2.2.1, aerobic degradation), but in very low quantities. However, a longer incubation time would have been more representative as fipronil itself show a low mobility in soil. Furthermore all 5 soil types don't vary significantly in pH (range is 6.1 to 6.9) and therefore an important demand of the OECD Guideline 312 is not fulfilled. Hence, no statement can be made to the pH-dependency of Fipronil for adsorption/desorption. According to OECD Guideline 312 a solution in distilled or deionised water (= artificial rain) containing 0.01M aqueous calcium chloride should be used.
Conclusion	Agree with the applicant's version
Reliability	2 The reliability indicator has been reduced due to the short incubation time.
Acceptability	Acceptable
Remarks	Error in Table A7.2.3.2-1 was corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.3 **Annex Point IIIA**	**Fate and behaviour in air**

Section 7.3.1 **Annex Point IIIA, VII.5**	**Phototransformation in air (estimation method)**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.3.1/01** Van der Gaauw, A. Estimation of the degradation of Fipronil by photo-oxidation in air (unpublished) (BASF Doc ID C011469)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Monograph 61, 1993	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Not applicable – Calculation	
		4. RESULTS	
4.1		The overall reaction rate constant of fipronil with OH-radicals was estimated to be K_{OH} 96.092 x 10^{-2} cm^3 $molecule^{-1}$ sec^{-1}. Using this value the half-life of fipronil was calculated as 0.111 day assuming a day is comprised of 12 sunlight hours and 0.056 day when considering a day of 24 sunlight hours	X
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	In the troposphere there are three important photochemical transformation processes that contribute to the degradation of a chemical, direct photo-reaction, indirect photo-reaction with OH-radicals and oxidation with ozone. In this study the rate constant for the atmospheric reaction between photochemically produced hydroxyl radicals and fipronil was estimated using the computer program OAPWIN, as developed by Atkinson	

Section 7.3.1 **Annex Point IIIA, VII.5**	**Phototransformation in air (estimation method)**

5.2	**Results and discussion**	The overall reaction rate constant of fipronil with OH-radicals was estimated to be K_{OH} 96.092 x 10^{-2} cm^3 $molecule^{-1}$ sec^{-1}. Using this value the half-life of fipronil was calculated as 0.111 day assuming a day is comprised of 12 sunlight hours and 0.056 day when considering a day of 24 sunlight hours. No further reactions of fipronil with OH-radicals need to be taken into account. Since the structure of fipronil contains no unsaturated carbon-carbon bonds necessary for reaction with ozone, it is not possible to predict any further reactions. Since fipronil has a low vapour pressure of 2 x 10^{-6} Pa it is very unlikely to be present in air	X
5.3	**Conclusion**	Although unlikely to reach the air due to a low vapour pressure value and due to application by soil incorporation or bait should fipronil reach the air, it will be rapidly degraded in the troposphere with a half-life of 0.111 day assuming 12 hours of sunlight	X
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	Agree with the applicant's version The overall reaction rate constant of fipronil with OH-radicals was estimated to be K_{OH} 96.092 x 10^{-12} cm^3 $molecule^{-1}$ sec^{-1}.
Conclusion	Using this value the half-life of fipronil was calculated as 0.167 day when considering a day of 24 sunlight hours.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.3.2 **Annex Point IIIA, XII.3**	**Fate and behaviour in air** 7.3.2 Further studies

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	No further studies were conducted, as Fipronil is not recommended as a fumigant.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Materials and methods	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4 **Annex Point IIA, VII**	**Effects on aquatic organisms**

General comment on studies summarized in this section. The database of aquatic studies conducted with fipronil is extensive (a references list of GLP regulatory aquatic studies is provided in Document IIA). Although the application method of the fipronil biocidal product Goliath Gel leads to negligible exposure in natural environments (see document IIIA-2.10), the list of studies summaries here have not been restricted to the core data set required by the Biocides directive (Acute fish toxicity, acute toxicity to invertebrates, Growth inhibition test on algae and Activated sludge). Since the database available for fipronil is much wider, summaries of several additional studies are included here. These additional studies (see section 7.4.3 below) follow the list of points included in point 7.4. (Chapter 3) of the document 'Data requirements for biocidal product types' version 4.3.1. April 2000 (Final Draft). It must be stressed that these additional studies are not strictly required by the Biocides Directive for fipronil as Goliath Gel, but are included voluntarily by the notifier to provide basic information for a better characterization of the toxicity profile of fipronil to freshwater aquatic organisms.

Section 7.4.1 **Annex Point IIA, VII**	**Aquatic toxicity, initial tests**

Section 7.4.1.1 **Annex Point IIA, VII.7.1**	**Acute toxicity to fish**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A.7.4.1.1/01** XXXX. Acute Toxicity to Bluegill, *Lepomis macrochirus*, under Flow-Through Test Conditions XXXX . (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: FIFRA Guideline 72-1	
2.2	**GLP**	Yes	

Section 7.4.1.1 **Annex Point IIA, VII.7.1**	**Acute toxicity to fish**	

2.3	**Deviations**	Yes – there were four deviations from the test protocol in the conduct of this study The first deviation was related of the size of the test fish. Due to availability, the bluegill which were tested averaged only 0.22g and were smaller than the 0.5 to 5.0g sized fish recommended in the test protocol. However these fish were actively feeding and are therefore, acceptable under FIFRA Guidelines The other deviations were related to water quality parameters. First, the hardness of the dilution water (56 mg/l as $CaCO_3$) was slightly below the range specified in the test protocol (i.e. 60 to 100 mg/l as $CaCO_3$). Second, the pH of the dilution water dropped to 7.1 on day 3 which is just below the lower limit set forth in the test protocol (i.e. 7.2 to 8.0). Lastly the water temperature ranged from 19.9 to 23.1°C during the 96-hour exposure. Only 1 hourly measurement (23.1°C) which occurred during the first 24 hours exceeded the protocol range of 21.0 to 23 °C. During the remainder of the test, the temperature range from 19.9 to 22.3°C. The lower limit of the temperature range (21.0°C) was exceeded periodically during the last 72 hours of exposure. None of the protocol deviations were significant and in the scientific judgement of the author these deviations did not affect the test results	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	100%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Analysis involved the extraction of an aliquot of water using hexane. The hexane was then collected, dried over sodium sulphate. Analysis was by Gas chromatography using an electron capture detector and an external standard.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	1.000g of fipronil was dissolved in 100ml dimethylformamide (DMF) See table A7.4.1.1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	

Section 7.4.1.1 **Annex Point IIA, VII.7.1**	**Acute toxicity to fish**

3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.1.1-2	
3.4.2	Test organisms	See Table A7.4.1.1-3	
3.4.3	Test system	See Table A7.4.1.1-4	
3.4.4	Test conditions	See Table A7.4.1.1-5	X
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and any abnormalities in behaviour or physical appearance	
3.4.7	Sampling	Test water was monitored daily (T, pH, O2)	
3.4.8	Monitoring of TS concentration	No	X
3.4.9	Statistics	Based on results of the test, the 24, 48 and 96 hour LC50 values and their 95 percent confidence limits were calculated using mean measured M&B 46030 concentrations. The LC50 values were estimated by a computer program using the following statistical methods; moving average angle, probit, logit and non linear interpolation. Confidence Limits for LC 50 values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined b the characteristics of the data (Stephan, 1977)	
		4. RESULTS	
4.1	**Limit test**	No	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	µg/l Nominal: 43 72 120 200 Actual: 19.9 42.4 74.5 108 186	X
4.2.2	Actual concentrations of test substance	µg/l Nominal: 43 72 120 200 Actual: 27.1 43.2 67.4 134 217 % of nominal: 104 11 94 112 108	X
4.2.3	Effect data (Mortality)	See Table A7.4.1.1-6	
4.2.4	Concentration / response curve	None available	

Section 7.4.1.1 **Annex Point IIA, VII.7.1**	**Acute toxicity to fish**

4.2.5	Other effects	See Table A7.4.1.1-6	
4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	See Table A7.4.1.1-6	
4.3.2	Nature of adverse effects	See Table A7.4.1.1-6	
4.4	**Test with reference substance**	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test material: fipronil purity 95.4% Test item : Juvenile Bluegill Sunfish (*lepomis macrochirus*), mean standard length 20mm (range 17 – 23); mean net weight 0.22g, starved over 48 hours prior to and during 96 hours of exposure. Dilution water purified tap water (hardness of 56 mg/l as $CaCO_3$), temperature range 19.9 – 23.1°C; oxygen content ≥7.2 mg/l, pH range 7.1 – 8.0. Exposure regime: 96 hours flow-through. Biological loading approx 0.15 g per litre and day. Test groups consisted of one replicate aquarium with 20 fish each per concentration. Nominal test concentrations based on preliminary testing 0 (control), 0 (solvent control, DMF at 20µl/l), 26, 43, 72, 120 and 200 µg/l. Mortality or abnormalities among the fish were monitored daily. The stock solution and exposure concentrations were analysed at the start and the end of the 96-hour exposure period. The LC50 was calculated with the moving average method.	**X**
5.2	**Results and discussion**	The actual concentrations were close to the nominal concentrations (94 – 112% of nominal). The results of the study were reported in terms of mean measured concentrations. Mortality and sublethal effects including loss of equilibrium and lethargy were observed at 67.4µl and above. TLC50 after 96 hours of exposure was calculated at 85.2µg/l, with 95% confidence limits of 74.2 – 99µg/l. Based on the absence of lethal of sublethal effects observed up to this concentration, the 96-hour NOEC was determined at 43.2 µg/l	
5.2.1	NOEC	43.2 µg/l	
5.2.2	LC_{50}	85.2µg/l, with 95% confidence limits of 74.2 – 99µg/l	
5.2.3	LC_{100}		
5.3	**Conclusion**		
5.3.1	Other conclusions		
5.3.2	Reliability	1	X
5.3.3	Deficiencies	None	

Table A7.4.1.1-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	No
Vehicle	Yes – dimethylformamide
Concentration of vehicle	1g in 100ml
Vehicle control performed	Yes
Other procedures	Not applicable

Table A7.4.1.1-2 Dilution water

Criteria	Details
Source	Jupiter, Florida Town water – carbon treated
Alkalinity	20 -24 mg/l
Hardness	56 mg/l as $CaCO_3$
pH	Not recorded
Oxygen content	Not recorded
Conductance	361 – 372 µmbos/cm
Holding water different from dilution water	No

Table A7.4.1.1-3 Test organisms

Criteria	Details
Species/strain	Juvenile bluegill *Lepomis macrochirus*
Source	XXXX
Wild caught	No
Age/size	Juvenile : 17 to 23 mm length. Weight 0.12 to 0.44 g
Kind of food	
Amount of food	
Feeding frequency	Daily
Pre-treatment	21 Days acclimatisation; Not fed during 48 hours prior to initiation of exposure
Feeding of animals during test	Not fed

Table A7.4.1.1-4 Test system

Criteria	Details
Test type	Flow through
Renewal of test solution	5 volume turnovers per day
Volume of test vessels	24 litres
Volume/animal	1.2l/fish
Number of animals/vessel	20
Number of vessels/ concentration	One
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.1-5 Test conditions

Criteria	Details
Test temperature	19.9 – 23.1°C
Dissolved oxygen	7.2 – 8.2 µg/l
pH	7.1 – 8.0
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	Not recorded
Photoperiod	16 hour light. 8 hour dark

Table A7.4.1.1-6 Mortality data

Test-Substance concentration measured [mg/l]	Cumulative Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Control	0	0	0	0	0	0	0	0
Solvent control	0	0	0	0	0	0	0	0
27.1	0	0	0	0	0	0	0	0
43.2	0	0	0	0	0	0	0	0
67.4	0	0a	2a	5a	0	0a	10a	25a
134	0a	7a	16a	19a	0a	35a	80a	95a
217	13	20	20	20	65a	100	1000	100

A indicates fish showing sublethal effects

Table A7.4.1.1-7 Validity Criteria for Acute Fish Test According to OECD Guideline 203

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	✓	
Concentration of dissolved oxygen in all test vessels > 60% saturation	✓	
Concentration of test substance ≥ 80% of initial concentration during test	✓	
Criteria for poorly soluble test substances	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 3, 2006
Materials and methods	The applicant's version is adopted with the following revisions: As the guideline study used is not suggested by the TNsG on Data Requirements, (E.C method C.1 or the corresponding OECD Guideline 203 are recommended), the study should be summarised with more details and full description of the method. 2.3 There are deviations/deficiencies from the OECD Guideline 203: - Temperature was slightly lower than the recommended range and has not been constant within a range of 2°C as recommended. - An alimentation period is recommended until 24 hours before the test is started and not 48 hours. - Light intensity is not specified. - Some parameters of dilution water are not indicated (oxygen content, pH). - Apparatus not indicated. However, it is considered that these deviations and reporting deficiencies are minor and did not affect the applicability of the results. 3.4.4 There is a mistake in the Table A7.4.1.1-5 on the oxygen content's unit: please change "*7.2 – 8.2 µg/l*" to "*7.2 – 8.2 mg/l*". 3.4.8 The exposure concentrations were analyzed at the start and the end of the 96-hour exposure period. 5.1 *Test material: fipronil purity* ~~95.4%~~ **100%.**
Results and discussion	The applicant's version is adopted with the following revisions: 4.2.1 This table must be deleted. 4.2.2 The following table presents the mean-measured concentrations at the start and the end of the 96-hour exposure period.

µg/l					
Nominal **concentration**	**26**	*43*	*72*	*120*	*200*
~~*Actual*~~ **Mean-measured concentration**	*27.1*	*43.2*	*67.4*	*134*	*217*
% of nominal	*104*	~~*11*~~**100**	*94*	*112*	*108*

The applicant doe not discuss the outcome of the study.

	It could be stated that measures (flow-through system with a short interval of renewal) have been taken to prevent abiotic degradation of TS. In addition it should be added that: *"the validity criteria according to OECD Guideline 203 can be considered as fulfilled (see table A.7.4.1.1-7)."* There is a mistake on the criteria of mortality of control animals is: ≤ 10% and not < 10%.
Conclusion	The applicant does not make any conclusions. With a 96 h-LC_{50} of 85.2 µg/L, it could be concluded that the tested substance Fipronil has a very high toxicological effect on the fish species Bluegill Sunfish.
Reliability	1
Acceptability	The study is considered acceptable as supportive to the risk assessment.
Remarks	

	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.1.2/01** XXXX. M&B 46030 -Acute toxicity to daphnids (*Daphnia magna*) during a 48-hour flow through exposure. XXXX (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA FIFRA 72-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	On day 1 of the study both min/max thermometers measured the minimum temperature of the exposure solutions as 18°C. The protocol states that temperatures will be maintained with 19 – 21°C. On test day 1, individual temperatures taken in each test vessel with a Brooklyn alcohol thermometer ranged from 19 – 20°C The protocol states that two quality assurance samples are to be prepared at each sampling interval at test material concentrations unknown to the analyst. During this study, three quality assurance samples were prepared by the analyst at test concentrations similar to the test concentration range	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	Batch no JW 20921/94	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	100%	
3.1.4	Composition of product	Not applicable – active substance	
3.1.5	Further relevant properties	Water solubility	X
3.1.6	Method of analysis	HPLC procedure according to the methodology given in Section 2. A method validation recover study conducted prior to the initiation of the definitive test established an average recovery of fipronil of 97.7 ± 8.84% from fresh water	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A.7.4.1.2-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A.7.4.1.2-2	
3.4.2	Test organisms	See table A.7.4.1.2-3	
3.4.3	Test system	See table A.7.4.1.2-4	
3.4.4	Test conditions	See table A.7.4.1.2-5	X
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	immobility	X
3.4.7	Sampling	Prior to the initiation of the test and on days 0 and 2	
3.4.8	Monitoring of TS concentration	As above	X
3.4.9	Statistics	EC50 Method of Stephan, 1977 –non-linear interpolation with 95% confidence limits	
		4. RESULTS	
4.1	**Limit test**	No	
4.2	**Results test substance**	Yes	
4.2.1	Initial concentrations of test substance	0 (control), 0 (solvent control), 47, 78, 130, 220 and 360 µg/l.	
4.2.2	Actual concentrations of test substance	Averaged at 82% of the nominal concentrations	X
4.2.3	Effect data (Immobilisation)	See table A7.4.1.2.1-6	X
4.2.4	Concentration / response curve	Not given in the report	
4.2.5	Other effects	None	
4.3	**Results of controls**	See Table A.7.4.1.2-6	
4.4	**Test with reference substance**	Not applicable	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: MB46030 of purity 100% Test organism: neonate *Daphnia magna* (<24 hours old), not fed during the test. Dilution water: fortified well water (hardness (160 -170 mg/l as $CaCO_3$), temperature range 19 – 21°C, oxygen content ≥ 7.7 mg/l, pH range 8.2 – 8.3. Exposure regime: flow through Test groups consisted of two replicate vessels with 10 daphnids each per concentration. Nominal test concentrations based on preliminary testing 0 (control), 0 (solvent control), 47, 78, 130, 220 and 360 µg/l. Immobilisation and abnormal behaviour or appearance of the daphnids were monitored daily. Exposure concentrations were sampled for analytical confirmation at the exposure initiation and termination. The 48 hour EC50 was estimated by non linear interpolation, the 95 confidence interval was calculated by binomial method.	
5.2	**Results and discussion**	The mean measured concentrations ranged 67 – 85% of nominal. The results of the study are summarised in terms of mean measured concentrations. Treatment related immobilisation and lethargy among the remaining daphnids at these levels were observed at 160 and 280 µg/l. The 48 hour EC50 was calculated at 190 µg/l with 95% confidence limits of 110 – 200 µg/l. The NOEC was determined at 52 µg/l.	X
5.2.1	EC_0	Not recorded	
5.2.2	EC_{50}	190 µg/l	
5.2.3	EC_{100}	Not achieved	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	As given above – it was the opinion of the study author that they did not alter the interpretation of the results or the conclusions drawn from this study.	

Table A.7.4.1.2-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	**Details**
Dispersion	Yes
Vehicle	acetone
Concentration of vehicle	90 ~~µg/l.~~ **µl/l**
Vehicle control performed	Yes
Other procedures	No

X

Table A.7.4.1.2-2 Dilution water

Criteria	Details
Source	Fortified well water
Alkalinity	120 µg/l.
Hardness	170 mg/l as $CaCO_3$
pH	8.2 – 8.3
Ca / Mg ratio	Not applicable
Na / K ratio	Not applicable
Oxygen content	Greater than 60% saturation
Conductance	500 µmhos/cm
Holding water different from dilution water	no

Table A.7.4.1.2-3 Test organisms

Criteria	Details
Species/strain	*Daphnia magna*
Source	XXXX
Age	<24 hours
Breeding method	Not recorded
Kind of food	Green algae and a trout food preparation
Amount of food	Not recorded
Feeding frequency	Daily
Pre-treatment	No
Feeding of animals during test	No

Table A.7.4.1.2-4 Test system

Criteria	Details
Renewal of test solution	6 solution volume replacements per day
Volume of test vessels	200 ml
Volume/animal	Not recorded
Number of animals/vessel	10 per vessel
Number of vessels/ concentration	2
Test performed in closed vessels due to significant	No

volatility of TS	

Table A.7.4.1.2-5 Test conditions

Criteria	Details
Test temperature	~~19 - 21°C~~ **18 - 21°C**
Dissolved oxygen	≥ 7.7 mg/l
pH	8.2 – 8.3.
Adjustment of pH	no
Aeration of dilution water	No
Quality/Intensity of irradiation	80 – 110 foot-candles
Photoperiod	16 hours light/8 hours darkness

X

Table A.7.4.1.2-6 Immobilisation data

~~Test-Substance concentration (nominal/effective)[‡] [mg/l]~~ **Mean measured Test-Substance concentration (µg/L)**	Immobile *Daphnia*				Oxygen [mg/l] 48h	pH 48 h	Temperature [°C] 48 h
	Number		Percentage				
	24 h	48 h	24 h	48 h			
280	0,0	8,9	0	85*	7.8	8.2	
160	0,0	2,5	0	35*	8.0	8.3	
110	0,1	0,0	0	0*	8.2	8.3	
52	0,0	0,1	5	5	8.3	8.3	~~19 - 20~~ **18 - 21**
34	0,0	1,1	0	10	8.3	8.3	
Solvent control	1,0	1,1	5	5	8.4	8.3	
Control	0,0	0,0	0	0	8.4	8.3	

X

~~[‡] measured concentrations~~

*** All of the surviving daphnids were observed to be lethargic**

Table A.7.4.1.2-8 Validity Criteria for Acute *Daphnia* Immobilisation Test According to OECD Guideline 202

	Fulfilled	Not fulfilled
Immobilisation of control animals < 10%	✓	
Control animals not staying at the surface	✓	
Concentration of dissolved oxygen in all test vessels > 3 mg/l	✓	
Concentration of test substance ≥ 80% of initial concentration during test	~~✓~~	Results based on mean-measured concentrations
Criteria for poorly soluble test substances	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 4, 2006
Materials and methods	The applicant's version is adopted with the following revisions: As the guideline study used is not suggested by the TNsG on Data Requirements, (E.C method C.1 or the corresponding OECD Guideline 203 are recommended), the study should be summarised with more details and full description of the method. 3.4.4 *Test conditions* - In accordance with the study report in Document IV, the intensity of irradiation reported in Table A.7.4.1.2-5 corresponds to the holding period and not to the test conditions, it is an intensity of 40-70 foot-candles for the test conditions. - Based on all temperature measurements performed during the test (measurements once daily in both replicate vessels of each treatment level and continuous in one replicate of the highest concentration tested) the exposure temperature range is 18- 21°C and not 19-20°C (correction has been done in Table A.7.4.1.2-5 and Table A.7.4.1.2-6). 3.4.6 *Immobility* and abnormal behaviour (e.g. erratic swimming behaviour, flared carapace). 4.2.2 For a better comprehension and better accuracy with the study report, the sentence " *averaged at 82% of the nominal concentrations*" should be changed to "Measured concentrations during the pre-test period (1 day prior to test initiation) averaged 82% of the nominal levels. However, analyses of the treatment levels (day 0 and 2) resulted in mean measured concentrations which averaged 74% of the nominal concentrations with a coefficient of variation which averaged 5.6%. Based on mean measured concentrations, the treatments levels were defined as 34, 52, 110, 160 and 280 µg/L". Additionally, in Table A.7.4.1.2-6 *Immobilisation data* the column title "*Test-Substance concentration (nominal/effective) [mg/l]*"should be changed to "Mean measured Test-Substance concentration (µg/L)". Indication of the corresponding standard deviation in parentheses will be informative.
Results and discussion	4.2.3 and 5.2 In accordance with the study report, the sentence "*Treatment related immobilisation and lethargy among the remaining daphnids at these levels were observed at 160 and 280 µg/l"* is not entirely correct and could be change by "At test termination, 85, 35, 0, 10 and 5 % of the daphnids were immobilised in the treatment levels of 280, 160, 110, 52 and 34 µg/L respectively and all of the surviving daphnids were observed to be lethargic in the three highest treatment levels (280, 160 and110 µg a.s./L)." For this last result, a footnote could be added in the Table A.7.4.1.2-6 *Immobilisation data*, like in the study report (Table 4). It should also be specified in the results that "no abnormal behaviour was observed among surviving daphnids in the two lowest treatment levels (52 and 34 µg/L) and in the control solutions (control with solvent and without solvent)."

	For a better accuracy, the sentence "results based on the measured concentrations" should be added in the Table A.7.4.1.2-8 for the validity criteria "Concentration of test substance ≥ 80% of initial concentration during test" . In addition it should be added in discussion that: *"* The validity criteria according to OECD Guideline 202 can be considered as fulfilled (see table A.7.4.1.2-8)."
Conclusion	The applicant's does not make any conclusions. The reported deficiency on the temperature range is considered of limited importance for the outcome of the study.
Reliability	As there is no evident concentration-effect relationship, the reliability indicator should be set to 2.
Acceptability	Acceptable as supportive data to the risk assessment.
Remarks	- Another study on acute toxicity to *Daphnia magna* not described in Doc IIIA but presented in the full data set shows a lowest EC_{50} (Ward and Rabe, 1989) ; the selection of this present study as key study for risk assessment was explained by the applicant: according to OECD guideline daphnids duration of exposure to the test substance in an acute test is 48 hours. The study of McNamara (1990; XXXX) was conducted according to OECD guideline 202 and therefore selected as key study. In the study of Ward and Rabe (1989; XXXX) daphnids were exposed to Fipronil for 96 hours, which is longer than allowed according to the OECD guideline 202. Further, daphnids were fed during the test, which is another deviation from the guideline.. - Errors in tables A.7.4.1.2-1, A.7.4.1.2-5 and A.7.4.1.2-6 were corrected in bold and underlined.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Note: The summary of one study with a species of insect is included because insects are considerably more sensitive to fipronil than daphnids.

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.1.2/02** XXXX (2003) Fipronil – Acute Toxicity to Mayfly Nymphs (*Hexagenia sp.)*under XXXX, (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes ASTM Guideline E-729	
2.2	**GLP**	Yes	
2.3	**Deviations**	The protocol states that the temperature of culture water will be maintained at 22± 1°C. During culturing, the temperature ranged from 22 to 24 °C during the final 24 hours before initiation. This deviation is not considered to have affected the results or the interpretation of the test.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Technical fipronil	
3.1.1	Lot/Batch number	BES1905	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	00.7% w/w	X
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	GC with electron capture detector,	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	0.0104 g of fipronil was dissolved in 100ml of acetone to prepare a stock solution. This solution was further diluted to prepare the nominal concentration solutions	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**

3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	The dilution water was a fortified well water and was characterized as having a total hardness and total alkalinity (as $CaCO_3$) of 170 and 120 mg/l respectively, a pH of 8.1 and a specific conductance of 500µmhos/cm	
3.4.2	Test organisms	*Hexagenia sp*., 60 day old nymphs, mean length 6.7 mm Frozen mayfly eggs were shipped to the performing laboratory and gradually warmed to test temperature in a shallow bowl containing fortified well water. The eggs were monitored daily for hatch. Following two days, hatch was observed and approximately 1000 newly-hatched nymphs were transferred to two rearing vessels. The rearing vessels (38-L aquaria) contained a 2 cm layer of artificial substrate covered with 24 litres of water. During holding and acclimatisation one half of the overlying water in each aquarium was renewed weekly by siphoning and replacing. During culture the mayflies were fed a finely-ground suspension of fish food, yeast and cerophyll.. Total body length, excluding cerci (caudal filaments) and wet weight was determined for a representative sample of the nymph population (N+20) used to initiate the test. The mean total length was 6.7mm (range 4.4 to 8.7mm) and mean wet weight was 0.0031g per nymph	X
3.4.3	Test system	The test was conducted in 1000ml glass beakers each containing 800ml of test solution and fine pieces of glass tube as an artificial substrate. Four replicates were maintained for each treatment level, the dilution water control and the solvent control.	
3.4.4	Test conditions	Test solution renewal at 48 hours of exposure, 21 to 22°C, illumination of 650 to 830 lux, photoperiod of 16 hours light and 8 hours darkness	
3.4.5	Duration of the test	96 hours the test was initiated when five mayfly nymphs (20 mayflies per treatment level and the controls) were selected impartially and placed in each replicate exposure vessel following test solution preparation. Mayflies were added one at a time until each exposure vessel contained one mayfly. This procedure was repeated until each vessel contained five mayflies. Based on mean wet weight of mayflies and test solution volume the loading concentration rate in each vessel was 0.019 of biomass per litre of test solution	
3.4.6	Test parameter	Mortality and sublethal effects. Death is defined as failure of the mayfly nymphs to respond by movement to gentle probing with a glass pipette	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**	
3.4.7 Sampling	The vessels were examined 0, 24,48,72 and 96 hours after initiation The pH, dissolved oxygen concentration and temperature were measured in each test aquaria at 0, 24, 48 (aged and new solutions), 72 and 96 hours of exposure. Temperature was continuously monitored in one vessel	
3.4.8 Monitoring of TS concentration	0, 48 (freshly prepared) and 96 hours	
3.4.9 Statistics	LC50 calculated by the computer program of Gulley et al., 1996	X
	4. RESULTS	
4.1 Limit test	No	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	Nominal concentrations: 0.063, 0.13, 0.25, 0.50 and 1.0µg a.i./L Measured concentrations: 0.064, 0.14, 0.23, 0.59 and 1.2µg a.i./L	
4.2.2 Actual concentrations of test substance	Nominal concentrations: 0.063, 0.13, 0.25, 0.50 and 1.0µg a.i./L Mean measured concentrations: 0.059, 0.14, 0.24, 0.52 and 1.1µg a.i./L	
4.2.3 Effect data	See Table A.7.4.1.2-15	X
4.2.4 Concentration / response curve		
4.2.5 Other effects	See Table A.7.4.1.2-15	X
4.3 Results of controls	See Table A.7.4.1.2-15	X
4.4 Test with reference substance	Not applicable	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Test substance fipronil with a purity of 99.7% Test organism: *Hexagenia sp*., 60 day old nymphs, mean length 6.7 mm Test condition: 96 hour duration, test solution renewal at 48 hours of exposure, 21 to 22°C, illumination of 650 to 830 lux, photoperiod of 16 hours light and 8 hours darkness. Nominal concentrations: 0.063, 0.13, 0.25, 0.50 and 1.0µg a.i./L Mean measured concentrations: 0.059, 0.14, 0.24, 0.52 and 1.1µg a.i./L	X
5.2 Results and discussion	The 96-hour LC_{50} value was calculated to be 0.44 µg a.i./L(corresponding 95% confidence intervals of 0.39 to 0.49 µg a.i./L). The No Observed Effect Concentration for this study was determined to be 0.14µg a.i./L	X
5.2.1 NOEC	0.14µg a.i./L	
5.2.2 LC_{50}	0.44 µg a.i./L	

Section 7.4.1.2 **Annex Point IIA, VII.7.2**	**Aquatic toxicity to invertebrates**

5.2.3 LC_{100}	1.1 µg a.i./L	
5.3 Conclusion		X
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A.7.4.1.2-9 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	**Details**
Dispersion	Dilution
Vehicle	Acetone
Concentration of vehicle	0.10mL/L
Vehicle control performed	Yes
Other procedures	None

Table A.7.4.1.10 Dilution water

Criteria	**Details**
Source	Fortified well water
Alkalinity	120 mg/L $CaCO_3$
Hardness	170 mg/L $CaCO_3$
pH	8.1
Ca / Mg ratio	Not recorded
Na / K ratio	Not recorded
Oxygen content	Not recorded
Conductance	500 µmhos/cm
Holding water different from dilution water	No

Table A.7.4.1-11 Test organisms

Criteria	Details
Species/strain	Mayfly (*Hexagenia rigida* and *Hexagenia limbata*)
Source	XXXX
Age	60 days
Breeding method	Raised from frozen eggs collected from female imagoes
Kind of food	20g flaked fish food, 15g yeas and 15g cerophyll blended together in 500ml deiozized water
Amount of food	10ml per 25 litre aquarium (500 nymphs)
Feeding frequency	weekly
Pre-treatment	No
Feeding of animals during test	No

Table A.7.4.1.2-12 Test system

Criteria	Details
Renewal of test solution	Once at 48 hours
Volume of test vessels	800ml
Volume/animal	160ml
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A.7.4.1.13 Test conditions

Criteria	Details
Test temperature	21 -22°C
Dissolved oxygen	7.4 to 9.1 mg/l
pH	7.7 – 8.0
Adjustment of pH	no
Aeration of dilution water	no
Quality/Intensity of irradiation	650 to 830 lux
Photoperiod	16 hours light 8 hours darkness

Table A.7.4.1.2-14 Immobilisation data

Mean measured Concentration (µg/litre)	Mean Cumulative Percentage Mortality [a]			
	24 hour	48 hour	72 hour	96 hour
Control	0(0)	0(0)	0(0)	10(2)
Solvent control	0(0)	0(0)	0(0)	10(2)
0.059	0(0)	0(0)	0(0)	0(0)
0.14	0(0)	0(0)	0(0)	0(0)
0.24	0(0)	0(0)	0(0) [c]	20(4)
0.52	0(0)	0(0)	0(0) [b]	75(15) [d]
1.1	0(0)	0(0)[b]	5(1)[d]	100(0)

[a] Actual number of mortalities is presented in parentheses
[b] Several of the surviving mayflies were lethargic
[c] Two of the surviving mayflies were lethargic
[d] All of the surviving mayflies were lethargic

Table A.7.4.1.2-15 Validity Criteria for Acute Invertebrate Immobilisation Test According to OECD Guideline 202

	Fulfilled	Not fulfilled
Immobilisation of control animals < 10%	✓	
Control animals not staying at the surface	✓	
Concentration of dissolved oxygen in all test vessels > 3 mg/l	✓	
Concentration of test substance ≥ 80% of initial concentration during test	✓	
Criteria for poorly soluble test substances	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 5, 2006
Materials and methods	Applicant's version is adopted with the following revision/amendment: 3.1.3 There is a mistake: purity of test substance is of 99.7% w/w and not 00.7% 3.4.2 It will be informative to add into the text paragraph: "*The mayflies used in this toxicity test were obtained as eggs from the University of Windsor, Ontario, Canada which were collected from female imagos of Hexagenia rigida and Hexagenia limbata."* In addition, some details should be added: *"During holding and acclimatisation one half of the overlying water in each aquarium was renewed weekly by siphoning and replacing. The temperature was 22 to 24°C throughout the holding period.Two weeks prior to test initiation, dissolved oxygen concentration and pH ranged from 7.4 to 8.7 mg/l and 7.5 to 7.8 respectively. During culture the mayflies were fed a finely-ground suspension of fish food, yeast and cerophyll. This food source was considered to be an acceptable quality since its periodical analyse for the presence of toxic metals, pesticides and PCBs."* 3.2 See Table A.7.4.1.2-9 3.4.1 See Table A.7.4.1.2-10 3.4.2 See A.7.4.1.2-11 3.4.3 See Table A.7.4.1.2-12
Results and discussion	Applicant's version is acceptable, however some points should be added in Section 5.2: - **Evaluation of Test conditions**: throughout the exposure, pH, dissolved oxygen concentration and temperature ranged from 7.7 to 8.0, 7.4 to 9.1 mg/L and 21 to 22°C respectively (see Table A.7.4.1.13). - **Analytical measurements**:mean measures concentrations ranged from 94 to 110% of the nominal fortified concentrations throughout the 96-hour exposure. Analysis of the quality control samples resulted in measured concentrations ranging from 90% to 113% of nominal fortified concentrations (0.06, 0.5 and 1µg a.i./L). The toxicity results are based on mean measured concentrations. - **Biological results**: The results of the observed mortality are summarized in the following table. No test item related mortality was observed up to 0.14 µg a.s./L. Mortality of 10% was observed in the control and solvent control at test termination. Mortality rates in the 0.24, 0.52 and 1.1 µg a.s./L test item concentrations were 20%, 75% and 100%, respectively. *The 96-hour LC_{50} value was calculated to be 0.44 µg a.i./L (corresponding 95% confidence intervals of 0.39 to 0.49 µg a.i./L). The No Observed Effect Concentration for this study was determined to be 0.14µg a.i./L".* 4.2.3, 4.2.5 and 4.3 ~~*Table A.7.4.1.2-15*~~ Table A.7.4.1.2-14 In addition it should be added that: *"In taking into account the validity criteria for Acute Invertebrate Immobilisation Test According to OECD Guideline 202, these validity criteria can be considered as fulfilled (see table A.7.4.1.2-15)."*

Conclusion	The applicant does not make any conclusions. With a 96 h-LC_{50} of 0.44 µg/L, it could be concluded that the tested substance Fipronil has a very high toxicological effect on the mayfly nymphs (*Hexagenia sp.*) and that this insect is considerably more sensitive to Fipronil than daphnids.
Reliability	1
Acceptability	Acceptable as supportive data to the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.1.3 **Annex Point IIA, VII.7.3**	**Growth inhibition test on algae**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.1.3/01** XXXX The algistatic activity of M&B 46030. 18 June 1991. (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EEC 87/302 OECD Guideline No. 201	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	**X**
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	
3.1.1	Lot/Batch number	78 GC 90	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	97%	
3.1.4	Composition of product	Active substance – not applicable	
3.1.5	Further relevant properties	n.a	
3.1.6	Method of analysis	Gas Chromatography, using an external standard	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Table A7.4.1.3-1	
3.3	**Reference substance**	None	
3.4	**Testing procedure**		
3.4.1	Culture medium	Standard nutrient medium	
3.4.2	Test organisms	Table A7.4.1.3-2	
3.4.3	Test system	Table A7.4.1.3-3	

Section 7.4.1.3 **Annex Point IIA, VII.7.3**	**Growth inhibition test on algae**	
3.4.4 Test conditions	Table A7.4.1.3-4	
3.4.5 Duration of the test	96-h	
3.4.6 Test parameter	biomass production, growth rate and appearance	
3.4.7 Sampling	0, 24, 48, 72 and 96 hours	
3.4.8 Monitoring of TS concentration	Chemical analysis of exposure concentrations was performed at 0 and 96 hours	
3.4.9 Statistics	graphic	
	4. RESULTS	
4.1 Limit test	No	
4.1.1 Concentration	Not applicable	
4.1.2 Number/ percentage of animals showing adverse effects	Not applicable	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	0 (control), 0 (solvent control 1% Tween 80 – acetone at 100µg/l), 10, 20, 40, 80 and 160 µg/l.	
4.2.2 Actual concentrations of test substance	0, 0,13.4 26.4, 47.1, 85.6 and 171 µg/l.	X
4.2.3 Growth curves		
4.2.4 Concentration / response curve		
4.2.5 Cell concentration data	A7.4.1.3-5	X
4.2.6 Effect data (cell multiplication inhibition)	A7.4.1.3-5	
4.2.7 Other observed effects	All test and control cultures were inspected at 96 hours. There were no abnormalities detected in any of the control or test cultures up to 40 µg/l. However at the test concentration of 80 µg/l the cells were observed to be clumped, and at 160 µg/l the cells were observed to be colourless, clumped and deformed in shape. The measured pH values in the test cultures increased slightly over the test period from pH 8.1 at initiation to pH 8.3 – 8.6 at termination	
4.3 Results of controls		X
4.4 Test with reference substance	No	
4.4.1 Concentrations	Not applicable	
4.4.2 Results	Not applicable	

Section 7.4.1.3 **Annex Point IIA, VII.7.3**	**Growth inhibition test on algae**

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item M&B 46030, purity > 95%. Test organisms *Scenedesmus subspicatus*. Growth medium: nutrient medium. Temperature 24°C; pH range 8.1 – 8.6, agitation at 100 rpm; continuous illumination at approx 7000 lux. Exposure regime: static exposure over 96 hours. Cell culture density was determined based on absorbance measurements at 665 nm at each 24 hour interval. Nominal test item concentration 0 (control), 0 (solvent control 1% Tween 80 – acetone at 100µg/l), 10, 20, 40, 80 and 160 µg/l. Each concentration consisted of 3 replicate vessels. Chemical analysis of exposure concentrations was performed at 0 and 96 hours. The 96-h EC50 values based on the area under the growth curve (AUC) and the maximum growth rate were determined graphically	
5.2	**Results and discussion**	The mean measured test substance concentrations averaged 119.5% of nominal at exposure initiation and 102.8% of nominal at the end of the test. The results of this study are summarised in terms of nominal concentration. Effect concentrations were determined in comparison to the solvent control. No effect on biomass production, growth rate or appearance of the algae cultures were observed at the test concentrations of 10, 20 and 40 µg/l. However, effects on both biomass production and maximum growth rate as well as abnormal appearance (colour/shape) of the algae were observed at 80 and 160µg/l.	**X**
5.2.1	NOE_rC	40 µg/l	
5.2.2	E_rC_{50}	74 µg/l	
5.2.3	E_bC_{50}	68µg/l	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.4.1.3-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	Yes
Vehicle	1% Tween 80 in acetone
Concentration of vehicle	160 mg test material in 10 ml
Vehicle control performed	Yes
Other procedures	None

Table A7.4.1.3-2 Test organisms

Criteria	Details
Species	*Scenedesmus subspicatus*
Strain	None given
Source	XXXX
Laboratory culture	Sterile nutrient medium was inoculated from a master culture and incubated under continuous illumination and aeration to give an algal suspension in log phase growth characterised by an absorbance of 0.082 (@ 665nm)
Method of cultivation	Not recorded
Pre-treatment	None
Initial cell concentration	1.08 x 10^5 cells/ml

Table A7.4.1.3-3 Test system

Criteria	Details
Volume of culture flasks	250 ml
Culturing apparatus	All flasks loosely stoppered, incubated and shaken (approximately 100 rpm) in an orbital shaker
Light quality	Continuous 7000 lux
Procedure for suspending algae	shaking
Number of vessels / concentration	3
Test performed in closed vessels due to significant volatility of TS	No

Table A7.4.1.3-4 Test conditions

Criteria	Details
Test temperature	24°C
pH	8.1 – 8.6
Aeration of dilution water	None: Gaseous exchange and suspension of algal cell maintained by orbital shaker
Light intensity	7000 lux
Photoperiod	continuous

Table A7.4.1.3-5 Cell concentration data

Test-Substance nominal concentration [~~mg/l~~ µg/L]	Area under curve at 96 hours	% inhibition	Growth rate (24 – 48 hours)	% inhibition
0 (control	16.73	-	0.028	-
0 Solvent control	16.40	-	0.027	-
10	16.40	0.0	0.027	<1.3>
20	17.38	<6.0>	0.026	2.4
40	16.63	<1.4>	0.030	<11.7>
80	5.56	66.1	0.017	35.5
160	3.58	78.2	0.004	86.7

<increase>

Table A7.4.1.3-6 Validity Criteria for Algal Growth Inhibition Test According to OECD Guideline 201

	Fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	✓	
Concentration of test substance ≥ 80% of initial concentration during test	✓	
Criteria for poorly soluble test substances	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 5, 2006
Materials and methods	2.3 There is a major deviation: The calculation of the E_rC_{50} was made from the average daily growth 24 – 48 hours. According to OECD Guideline 201, the test endpoint is inhibition of growth expressed as average growth rate over the test duration (normally days 0-3) while the log-biomass integral i.e. area under the growth curve is considered as an additional endpoint.
Results and discussion	As see above, the results test report on the calculated test endpoint is not consistent with OECD guideline 201. 4.2.2 Actual concentrations of test substance ~~0,~~ 0, 13.4~~.~~ 26.4, 47.1, 85.6 and 171 µg/l. 4.2.5 There is a mistake in the Table A7.4.1.3-5 "*Cell concentration data"* on the Test-Substance nominal concentration's unit: please change mg/L to µg/L 4.3 Results of controls should be provided, as e.g.: "*the mean cell density of control and solvent control at 96h is of 1.97 x 10^6 cells/ml and 1.79 x 10^6 cells/ml respectively."* In addition it should be added that: *"The validity criteria according to OECD Guideline 201 can be considered as fulfilled (see table A.7.4.1.3-6)."*
Conclusion	With the performed calculation of growth rate and at the sight of the absorbance values results, the E_rC_{50} is most likely underestimated.
Reliability	In absence of relevant calculated test endpoint E_rC_{50} over the exposure period, the reliability indicator should be set to 2.
Acceptability	NOEC and E_bC_{50} endpoints are acceptable as supportive data.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.1.4 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.1.4/01** Herti, J. (2001) Toxicity of EXP60720A to Activated Sludge in a Respiration Inhibition Test. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 209	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	EXP 60720A	
3.1.1	Lot/Batch number	OP990918	
3.1.2	Specification	A water dispersible granule	
3.1.3	Purity	788g/kg	X
3.1.4	Further relevant properties	None	
3.1.5	Method of analysis	Not applicable	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable the formulation is fully dispersible in water	
3.3	**Reference substance**	3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Culture medium	Not applicable	

Section 7.4.1.4 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**	
3.4.2 Inoculum / test organisms	Activated sludge, mirco organisms from a domestic waste water treatment plant (Groß-Zimmern, Germany). The sludge was washed by centrifugation and the supernatant liquid phase was decanted. The solid material was re-suspended in tap water and again centrifuged. The latter procedure was repeated twice. An aliquot of the final sludge suspension was weighed, dried and the ratio of wet sludge (g) to its dry weight (g) determined. Based on this ratio, calculated aliquots of washed sludge suspension, corresponding to 3g dry material per litre, were made up with tap water. To this mixture, 50ml synthetic swage feed per litre was added one day prior to use, and the sludge was kept at room temperature under continuous aeration until use. The pH of the activated sludge was determined to be 7.5	
3.4.3 Test system	Glass flasks of approximately 1 litre volume and Karlsruher flasks of 250ml volume. Each test unit was uniquely identified with the study number treatment and replicate number.	
3.4.4 Test conditions	Controlled environment room: temperature 21 – 22°C with aeration at approximately 0.6 litre per minute	
3.4.5 Duration of the test	3 hours	
3.4.6 Test parameter	Oxygen concentration after 3 hours Oxygen consumption (mg 0_2 l^{-1}) for following 10 minutes after aeration is stopped pH	
3.4.7 Analytical parameter	None	
3.4.8 Sampling	At test termination	
3.4.9 Monitoring of TS concentration	None	
3.4.10 Controls	No test solution or reference solution	
3.4.11 Statistics		
	4. RESULTS	
4.1 Preliminary test	None conducted	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	10, 32, 100, 320 and 1000 mg active substance per litre	
4.2.2 Actual concentrations of test substance	Not measured	
4.2.6 Effect data	The influence of EXP60720A on the respiration rate of activated sludge is presented in Table A7.4.1.4-1	
4.2.7 Other observed effects	None	

Section 7.4.1.4 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**

4.3	**Results of controls**	Presented in Table A7.4.1.4-1	
4.4	**Test with reference substance**		
4.4.1	Concentrations	3.2, 10, 32 mg/l	
4.4.2	Results	Presented in Table A7.4.1.4-2	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of the test item (EXP 60720A), a water dispersible granule formulation containing a nominal 800 g/kg fipronil, on the activity of activated sludge was evaluated by measuring the respiration rate under defined conditions. The respiration rate (oxygen consumption) of an aerobic activated sludge fed with a standard amount of synthetic sewage was measured in the presence of various concentrations of the test item after an incubation period of 3 hours. The concentrations used were 10, 32, 100, 320 and 1000 mg/l based on the concentration of the active ingredient fipronil; 3.2, 10 and 32 mg/l 3,5-dichlorophenol and two inoculum controls	X
5.2	**Results and discussion**	In comparison to the inoculum controls the respiration rate of the activated sludge was not inhibited (-5.4 to 0.3%) up to the highest test concentration of 100 mg active ingredient/l. Test item concentrations exceeding 1000 mg a.i/litre were not tested	X
5.2.1,	5.2.2, 5.2.3 $EC_{20,}$ $EC_{50,}$ EC_{80}	Based on measured inhibition rates, the 3-hour $EC_{20,}$ EC_{50} and EC_{80} could not be quantified because up the highest nominal test concentration of 1000 mg a.i/l less than 20% inhibition was noted after three hours incubation. Nevertheless, the 3-hour $EC_{20,}$ EC_{50} and EC_{80} are clearly higher than 1000 mg a.i/litre under the test conditions.	
5.3	**Conclusion**		X
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.4.1.4-1 Influence of EXP60720A on oxygen consumption of activated sludge

Treatment	Concentration (mg a.i/l)	Oxygen consumption (mg O_2/l min)	Inhibition (%)	pH values		Oxygen concentration (mg O_2/l)	
				Start*	End*	Start*	End*
control		0.360	-	7.5	8.6	7.1	7.2
control		0.421	-	7.5	8.5	6.3	6.7
Mean % deviation		0.391 14.5					
Test item	1000	0.400	-2.3	7.5	8.3	6.5	6.5
Test item	320	0.412	-5.4	7.5	8.3	6.5	6.5
Test item	100	0.411	-5.1	7.4	8.4	6.2	6.8
Test item	32	0.400	-2.3	7.4	8.4	6.2	6.5
Test item	10	0.390	0.3	7.5	8.4	6.1	6.6

* start and end of hour aeration

Table A7.4.1.4-2 Influence of 3,5-Dichhlorophenol (DCP) on oxygen consumption of activated sludge

Treatment	Concentration (mg a.i/l)	Oxygen consumption (mg O_2/l min)	Inhibition (%)	pH values		Oxygen concentration (mg O_2/l)	
				Start*	End*	Start*	End*
control		0.360	-	7.5	8.6	7.1	7.2
control		0.421	-	7.5	8.5	6.3	6.7
Mean % deviation		0.391 14.5					
3,5-DCP	32	0.032	91.8	7.5	8.6	6.5	8.4
3,5-DCP	10	0.167	56.5	7.5	8.6	6.3	7.7
3,5-DCP	3.2	0.219	43.7	7.5	8.7	6.2	7.9

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 6, 2006
Materials and methods	Applicant's version is acceptable with the following amendment : 3.1.3 Purity : *788 g/kg* (for the formulated product).
Results and discussion	5.2 It should be added that: *"the validity criteria according to OECD Guideline 209 can be considered as fulfilled i.e. 1) the respiration rate of the two controls differed by 14.5% and thus not differed by more 15% and 2) the 3-hours EC50 of the reference substance 3,5-Dichlorophenol was determined to be 5.1 mg/L and is thus in the range of 5 to 30 mg/L."*
Conclusion	The applicant does not make any conclusions. It could be concluded that: "*under the conditions of the test, the test substance EXP 60720A (Fipronil 788 g/kg) has no effect on the respiration rate of the activated sludge up to the maximum concentration tested (1000 mg/L)."*
Reliability	1
Acceptability	Acceptable as supportive data to the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.2 **Annex Point IIA, VII.7.5**	**Bioconcentration**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.2/01** XXXX. (1992) [^{14}C]-M&B 46030: Bioaccumulation Test in Bluegill Sunfish. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA N, 165-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	**X**
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	GHS707A	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	98.5%	
3.1.4	Further relevant properties	Not applicable	
3.1.5	Radiolabelling	14C-labelled M&B 46030 Specific activity 43.86µCi/mg	
3.1.6	Method of analysis	Reverse phase HPLC with UV detection	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing/estimation procedure**		
3.3.1	Test system / performance	Test item: [14C]-labelled M&B 46030, purity 98.5, specific activity 43.86µCi/mg. Test organisms: Bluegill Sunfish (*Lepomis macrochirus*), weight range 0.666 – 3.664g and length range 30 – 45 mm. Provided by Osage Catfisheries, Missouri, USA.	

Section 7.4.2 **Annex Point IIA, VII.7.5**	**Bioconcentration**	
	Test design: continuous flow-through over 35 days (uptake phase), then transfer for 14 days in untreated medium in continuous flow-through (depuration phase). Test concentrations 0 (control), 0.85µg/l. The target concentration of 0. 85µg/l. was selected because (a) it is corresponding to 1/100 of the 96-h value in Bluegill Sunfish, (b) the test material is soluble in water at this concentration, and (c) the concentration is adequate for analytical detection. Dilution water: charcoal-filtered, dechlorinated tap water, temperature range 20.9 – 22.8°C, pH range 7.27 – 7.83. Fish were maintained and tested under continuous flow conditions in glass tanks of 90 litres capacity which were enclosed by Perspex lids to minimise contamination by dust. Total flow rates through each tank were recorded daily and provided for over 5 tank volume changes every 24 hours. Fish were fed daily with commercial salmon diet. Holding tanks were cleaned daily to remove excess food and other particular organic matter. The tank for the test item group contained initially 158 fish and the control contained 147. The radio labelled test substance was added to the test tank 3 days prior to adding fish to the tank, to allow equilibration of the test material within the continuous flow system. The fish were observed daily for signs of disease, stress, irritation and other effects. The pH, temperature range, conductivity and dissolved oxygen concentration were measured daily in each tank. Total hardness, alkalinity and un-ionised ammonia in each tank were measured at weekly intervals. On each of the days 0 (prior to exposure) 3, 7, 14, 21, and 28 of the uptake phase and on days 1, 3, 7, 10 and 14 of the depuration phase, 10 fish were sampled from each group for length and weight measurement and subsequent determination of total radioactivity in whole fish, in edible parts (fillet) and in non-edible fractions (viscera) Water samples were taken daily form each tank and analysed for total radioactivity. Analytical results were used to establish the apparent steady state phase for bioaccumulation and the bioconcentration factor. Additional analysis on the nature of the radioactivity was performed. Concentrations of total radioactivity and bioconcentration factor in whole fish and tissue fractions were analysed for homogeneity of variance using the F-max test. If the group variances appeared homogeneous a parametric ANONA was used and comparisons between Day 35 and Days 3, 7, 14, 21 and 28 of the uptake phase made using Dunnett's test. All tests were 2-tailed and 5% significance level was used throughout. If variances were heterogeneous, log or square root transformations were used to stabilise variances	**X**

Section 7.4.2 **Annex Point IIA, VII.7.5**	**Bioconcentration**	
	The Bioconcentration Factor (BCF) defined as the quotient, at a given time point during the uptake phase of a Bioconcentration test, of the concentration of a material in tissues of an aquatic organism divided by the effective average exposure concentration in the solution containing that organism.	**X**
3.3.2 Estimation of bioconcentration	The Bioconcentration Factor (BCF) defined as the quotient, at a given time point during the uptake phase of a Bioconcentration test, of the concentration of a material in tissues of an aquatic organism divided by the effective average exposure concentration in the solution containing that organism.	
	4. RESULTS	
4.1 Experimental data		
4.1.1 Mortality/ behaviour	None seen	
4.1.2 Lipid content	Not given	
4.1.3 Concentrations of test material during test	810 – 990 ng equiv.1^{-1} (mean 900 ng equiv.1^{-1}) during the uptake phase. Analysis of water samples using reversed phase HPLC confirmed results from analysis of radioactivity, a mean test concentration of 76.5 was determined during the uptake and the test material could not be detected during the depuration phase or in the control experiment	X
4.1.4 Bioconcentration factor (BCF)	The apparent steady state BCF for radioactivity from [14C-fipronil in whole fish was 321. Uptake residues were rapidly and nearly completely (>99%) eliminated from whole fish within 14 days.	X
4.1.5 Uptake and depuration rate constants		
4.1.6 Depuration time		
4.1.7 Metabolites	Not applicable	
4.1.8 Other observations	None reported	
4.2 Estimation of bioconcentration		X
	5. APPLICANT'S SUMMARY AND CONCLUSION	

Section 7.4.2 **Annex Point IIA, VII.7.5**	**Bioconcentration**

5.1	**Materials and methods**	Test item: [14C]-labelled M&B 46030, purity 98.5, specific activity 43.86µCi/mg. Test organisms: Bluegill Sunfish (*Lepomis macrochirus*), weight range 0.666 – 3.664g and length range 30 – 45 mm. Test design: continuous flow-through over 35 days (uptake phase), then transfer for 14 days in untreated medium in continuous flow-through (depuration phase). Test concentrations 0 (control), 0.85µg/l. The target concentration of 0. 85µg/l. was selected because (a) it is corresponding to 1/100 of the 96-h value in Bluegill Sunfish, (b) the test material is soluble in water at this concentration, and (c) the concentration is adequate for analytical detection Dilution water: charcoal-filtered, dechlorinated tap water, temperature range 20.9 – 22.8°C pH range 7.27 – 7.83 Fish were fed daily with commercial salmon diet. Holding tanks were cleaned daily to remove excess food and other particular organic matter. The tank for the test item group contained initially 158 fish and the control contained 147. On each of the days 0 (prior to exposure) 3, 7, 14, 21, and 28 of the uptake phase and on days 1, 3, 7, 10 and 14 of the depuration phase, 10 fish were sampled from each group for length and weight measurement and subsequent determination of total radioactivity in whole fish, in edible parts (fillet) and in non-edible fractions (viscera). Water samples were taken daily form each tank and analysed for total radioactivity. Analytical results were used to establish the apparent steady state phase for bioaccumulation and the bioconcentration factor. Additional analysis on the nature of the radioactivity was performed and reported in a separate study.	
5.2	**Results and discussion**	Analysis of total radioactivity and HPLC analysis showed the actual concentration during the uptake phase close to nominal. Statistically, the apparent steady state in whole fish was observed within 7 days.	X
		On the first day of the depuration phase, 6% of the nominal concentration was measured in the dilution water of the treatment group; afterwards concentrations were below QD. The concentration of total radioactivity in whole fish decreased by 57% during 3 days, by 90% during 7 days, and by over 99% during 14 days of depuration.	
		The uptake kinetics were considered to approach a simple 2-compartment model with measured BCF at steady state close to theoretical values predicted based on the log P_{ow}. Depuration was rapid and nearly complete (99%) within 14 days.	**X**
5.3	**Conclusion**	The apparent steady state BCF for radioactivity from [14C-fipronil in whole fish was 321. Uptake residues were rapidly and nearly completely (>99%) eliminated from whole fish within 14 days.	
5.3.1	Reliability	1	

Section 7.4.2 **Annex Point IIA, VII.7.5**	**Bioconcentration**

5.3.2 Deficiencies	Not applicable	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 6, 2006
Materials and methods	Applicant's version is adopted with the following revisions: 2.3 According to the OECD Guideline 305, fishes should be exposed to at least two concentrations of the test substance. However this deviation is not considered to have affected the results or the interpretation of the test. Moreover, this guideline recommended for substances with high lipophilicity (i.e. with log Kow>3) to express bioconcentration in relation to lipid content in addition to whole body weight. Indeed, test results show variability between fish which will be from in part to the relationship between the lipid content of the test fish and the observed bioconcentration. 3.3.1 *The radio labelled test substance was added to the test tank 3 days prior to adding fish to the tank, to allow equilibration of the test material within the continuous flow system.* Total radioactivity was examined daily in water during the test. Analysis of water samples was also carried out weekly with reversed-phase HPLC.
Results and discussion	Applicant's version is adopted with the following revisions: **4.1.3 Concentrations of test material during test**: 0.81 – 0.99 µg.1^{-1} *(mean* 0.9 µg.1^{-1}*) during the uptake phase. Analysis of water samples using reversed phase HPLC confirmed results from analysis of radioactivity, a mean test concentration of* 0.765 µg.1^{-1} *was determined during the uptake and the test material could not be detected during the depuration phase or in the control experiment.* 4.1.4 and 5.2 It will be consistent with the testing procedure to report the BCF in whole fish, in edible parts (fillet) and in non-edible fractions (viscera) with the corresponding concentration in the fish/specified tissues. 4.2 According to the TGD Part II Section 3.8.3.2, BCF for fish can be predicted from the relationship between log K_{ow} and BCF: $\log BCF_{fish} = 0.85 \times \log K_{ow} - 0.70$ Thus for a log K_{ow} of 3.5 and for a log K_{ow} of 4 (as reported in part IIA 3.9), the corresponding calculated BCF is 188 and 501 respectively. 5.2 This part should be revised as follows: - The results should be indicated in a table :

Day N°	Bioconcentration factor		
	Edible fraction	Inedible fraction	Whole fish
3	142	390	200
7	87	181	250
14	155	482	278
21	184	612	354
28	156	574	272
35	159	631	380
	Apparent steady-state BCF		
Extend (Day N°)	14-35	14-35	14-35
Mean BCF	164	575	321
Standard deviation	14	66	54

- The last paragraph should be amended as follows: "*A single pattern of accumulation of [14C]-fipronil in Bluegill Sunfish was observed during the test which indicates that uptake kinetics approach a simple 2-compartment model. The mean measured BCF of 164 for edible tissue at steady state is close to the calculated value of 187 based on the log K_{ow} of 3.5 (according to the TGD Part II Section 3.8.3.2). In all tissues and whole fish, depuration was rapid and nearly complete (96%) within 14 days.*"

Conclusion Agree with the applicant version.

Reliability 1

Acceptability Acceptable as supportive data to the risk assessment.

Remarks

COMMENTS FROM …

Date

Results and discussion

Conclusion

Reliability

Acceptability

Remarks

Section 7.4.3 **Annex Point IIIA, XIII.2**	**Effects on aquatic organisms, further studies**

Section A7.4.3.1 **Annex Point IIIA, XIII.2.1**	**Prolonged toxicity to an appropriate species of fish**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [x]**	
Detailed justification:	In addition to negligible exposure, study XXXX (summarized in section, 7.4.3.2.) also covers this point because newly-hatch fish were exposed continuously to fipronil for 60 days (prolonged exposure) and the endpoints assessed included lethal and sublethal toxicity.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 10, 2006
Evaluation of applicant's justification	Agree with applicant's justification: the environmental exposure of fipronil from the intended uses of Goliath Gel is assessed negligible and does not indicate a risk to the environment, therefore it is not considered necessary to submit further testing.
Conclusion	Applicant's justification is acceptable
Remarks	A quantitative exposure assessment on the service life stage will be informative
	COMMENTS FROM …
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section 7.4.3.2 **Annex Point IIIA, XIII.2.2**	**Effects on reproduction and growth rate on an appropriate species of fish**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A.7.4.3.2/01** XXXX. (M&B 46030) – The toxicity to rainbow trout (*Oncorrhynchus mykiss*) during an early life-stage exposure) (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	USEPA Guideline 72-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	Lot 78 GC90	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	96.7	X
3.1.4	Composition of product	Technical material	
3.1.5	Further relevant properties	none	
3.1.6	Method of analysis	Not recorded	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A 15.4 mg/l stock solution was prepared by diluting approximately 0.796g fipronil with acetone to 50 ml. The resulting stock solution was clear and colourless.	X
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	Aerated well water	X

Section 7.4.3.2 **Annex Point IIIA, XIII.2.2**	**Effects on reproduction and growth rate on an appropriate species of fish**	
3.4.2 Test organisms	Unfertilised rainbow trout eggs and sperm supplied by XXXX	
3.4.3 Handling of embryos and larvae (OECD 210/212)	After fertilisation the embryos were allowed to remain undisturbed in control water for approximately I hour.	
3.4.4 Test system	Aquaria measuring 39 x 20 x 25cm with a 19.5cm high drain that maintained a constant exposure solution volume of 15l.	X
3.4.5 Test conditions	See Table A7.4.3.2-5	X
3.4.6 Duration of the test	90 days	X
3.4.7 Test parameter	Hatching, survival, total length and wet weight	
3.4.8 Sampling	At test end	X
3.4.9 Monitoring of TS concentration	Day 0 and weekly thereafter	
3.4.10 Statistics	William's, Dunnett's or Bonferroni's tests	
	4. RESULTS	
4.1 Range finding test		
4.1.1 Concentration	25 -1.6 26 µg/l	X
4.1.2 Number/ percentage of animals showing adverse effects	Survival was reduced at 1226 µg/l and above Statistical analysis failed to demonstrate a significant reduction in wet weight or total length at any concentration	X
4.1.3 Nature of adverse effects	Survival rate reduced	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	100, 50, 25, 12 and 6.226 µg/l	
4.2.2 Actual concentrations of test substance	60, 26, 15,6.6 and 2.6 µg/l	
4.2.3 Effect data	See Table A7.4.3.2-8	
4.2.4 Concentration / response curve	None observed	
4.2.5 Other effects	None observed	
4.3 Results of controls		
4.3.1 Number/percentage of animals showing adverse effects	See Table A7.4.3.2-8	
4.3.2 Nature of adverse effects	See Table A7.4.3.2-8	

Section 7.4.3.2 **Annex Point IIIA, XIII.2.2**	**Effects on reproduction and growth rate on an appropriate species of fish**	
4.4 **Test with reference substance** 4.4.1 Concentrations 4.4.2 Results	Not applicable	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 **Materials and methods**	The purpose of this study was to determine the MATC (Maximum Acceptable Toxicant Concentration of fipronil during an early life-stage exposure with rainbow trout *(Oncorhynchus mykiss)*. Rainbow trout were exposed continuously for 90 days (60 days post-hatch) to nominal concentration of 100, 50, 25, 12 and 6.2 µg/l fipronil, a dilution water control and a solvent (acetone) control. Analyses of the exposure solutions during the exposure period resulted in mean measured concentrations of 60, 26, 15,6.6 and 2.6 µg/l. Observations were made on embryo viability, survival of organisms at hatch and survival and growth (total length and wet weight) of larvae after 60 days post hatch exposure.	
5.2 **Results and discussion**	Embryo viability in all concentrations of fipronil tested ranged from 92 to 97% and was statistically comparable to the viability of pooled control organisms (94%). Survival of organisms at the completion of the hatching period (test day 30) in all tested concentrations ranged from 97 to 100%. Statistical analysis demonstrated that there were no treatment level effects for this biological endpoint. At test termination (60 days post-hatching), no surviving larvae were observed in the exposure solutions of the highest mean measured concentration of fipronil tested (60µg/l) while survival of 78% was observed among organisms exposed to the 26 µg/l treatment level. Statistical analysis demonstrated that survival was significantly reduced in these two exposure solutions when compared to the pooled control (98% survival). Survival ranging from 93 to 98% was recorded in the remaining test concentrations (15 – 2.6µg/l) Statistical analysis demonstrated no significant reduction in survival at concentrations ≤ 15µg/l as compared to the survival of pooled control organisms. Since the percent survival of the rainbow trout exposed to the two highest mean measured concentration of fipronil tested was significantly affected by exposure to fipronil, growth data for these treatment levels was excluded from further statistical analysis.	

Section 7.4.3.2 **Annex Point IIIA, XIII.2.2**	**Effects on reproduction and growth rate on an appropriate species of fish**	
	The mean total length of larvae exposed to the 15 and 2.6µg/l treatment levels was 58mm and was significantly reduced ($p \leq 0.05$) as compared to the mean total length of pooled control larvae (60mm). The mean total length of larvae in the 6.6 treatment level was 60mm and was comparable to the performance of pooled control organisms. Although a statistical difference in larval length was demonstrated by the William's Test both the lowest treatment level (2.6µg/l) and at the 15µg/l treatment level, this difference was not considered to be of biological significance. The absence of a concentration-response relationship, for organism length, at these lower treatment levels and no statistical difference between larval weight at the same exposure concentrations, corroborates the lack of biological significance between the growth of rainbow trout exposed to ≤ 15µg/l fipronil and the control solutions. The mean wet weight of organisms exposed to the 15, 6.6 and 2.6 µg/l treatment levels ranged from 2.1 to 2.2g and was statistically comparable to the mean wet weight of the pooled control organisms (2.2g). Based on these data, it was established that larval survival was the most sensitive indicator of the toxicity of fipronil to rainbow trout. The Lowest Observed Effect Concentration (LOEC) for this study was determined to be 26 µg/l and the No Observed Effect Concentration (NOEC) was determined to the 15 µg/l. The MATC was estimated to be ≤ 15 and ≤ 26µg/l (geometric mean MATC = 20g/l).	
5.2.1 NOEC	15 µg/l	
5.2.2 LOEC	26 µg/l	
5.3 Conclusion		
5.3.1 Other conclusions	None	
5.3.2 Reliability	1	
5.3.3 Deficiencies	None	

Table A7.4.3.2-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	
Vehicle	Acetone
Concentration of vehicle	
Vehicle control performed	Yes
Other procedures	None

Table A7.4.3.2-2 Dilution water

Criteria	Details
Source	Well
Salinity	Not recorded
Hardness mg/l as $CaCO_3$	~~30~~ **24 32**
pH	~~6.6 – 7.4~~ **6.8 – 7.4**
Oxygen content (mg/l)	10
Conductance (µmhos/cm3)	~~120~~ **100 - 150**
Holding water different from dilution water	Not different

X

Table A7.4.3.2-3 Test organisms

Criteria	Details
Species/strain	Oncorhynchus mykiss
Source	Mount Lassen Trout Farm California USA
Wild caught	No
Age/size	Unfertilised egg and sperm
Kind of food	Not applicable
Amount of food	Not applicable
Feeding frequency	Not applicable
Post-hatch transfer time	Not applicable
Time to first feeding	13 days post hatch
Feeding of animals during test	Live brine shrimp – twice daily from the swim up stage
Treatment for disease within two weeks preceeding test	Not applicable

Table A7.4.3.2-4 Test system

Criteria	Details
Test type	Flow through
Renewal of test solution	6.6 aquarium volume replacements in 24 hours
Volume of test vessels	15l
Volume/animal	150ml
Number of animals/vessel	100
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.3.2-5 Test conditions

Criteria	Details
Test temperature	12 ± 1°C
Dissolved oxygen	~~10~~ **9.6 – 10** mg/l
pH	6.6 – 7.4
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	30 – 50 Footcandles
Photoperiod	16 hours light, 8 hours dark

X

Table A7.4.3.2-6 Validity Criteria for Fish Tests According to OECD Guidelines 210/212

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	✓	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	✓	
Overall survival of fertilised eggs in controls (and solvent controls) ≥ value, specified for the specific test species	✓	
Test substance concentrations maintained within ± 20% of mean measured values	✓	
No effect on survival nor any other adverse effect found in solvent control	✓	
Further criteria for poorly soluble test substances		

Table A7.4.3.2-7 Validity Criteria for Fish Test According to OECD Guidelines 215

	Fulfilled	Not fulfilled
Concentration of dissolved oxygen in all test vehicles > 60% saturation	✓	
Difference of water temperature < 1°C between test chambers at any time during test; temperature within range of 2°C of the temperature for specific test species	✓	
Mortality of control animals < 10%	✓	
Increase of fish weight sufficient for detection of the minimum variation of growth rate considered as significant	✓	

Table A7.4.3.2-8 Table of Effects

Mean measured concentration (µg/l)	Mean embryo viability (%)	Mean survival at hatch (%)	Mean larval survival (%)	Mean total length + SD (mm)	Mean wet weight + SD (g)
0 (control)	94	98	98	61 ± 5.3	2.2 ± 5.3
0 (solvent control)	94	98	98	60 ± 2.6	2.1 ± 2.7
0 (pooled control)	94	98	98	60 ± 1.0	2.2 ± 0.08
2.6	94	98	98	58 ± 4.5*	2.1 ± 3.4
6.6	92	99	98	60 ± 2.5	2.1 ± 3.1
15	94	100	93	58 ± 3.5*	2.1 ± 3.9
26	97	98	78*	50 ± 9.1(*)	1.7 ± 5.7(*)
60	95	97	0*	-	-

(*) sublethal effect data from concentrations with significant mortality data were excluded from statistical analysis

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 10, 2006
Materials and methods	Agree with the applicant's version with the following amendments: 3.1.1 Purity : *96.7* % 3.1.6 A HPLC method is indicated in the study report. 3.2 It could be indicated that "*the solvent control contained the maximum amount of acetone present in any test concentration i.e. 6.5 µL/L".* 3.4.1 There are some mistakes/inaccuracy in the Table A7.4.3.2-2: - Hardness mg/l as $CaCO_3$: *24 - 32* - pH range was *6.8 - 7.4* and not *6.6 – 7.4* - Conductance: *100 - 150* 3.4.4 Some points need revision/amendment in the Table A7.4.3.2-4 *Test system:* - Number of animals/vessel and Volume/animal: the test was performed in two major stages (pre and post-hatching) with a different number of animals / vessel between them (there was 100 eggs at the start of test and 20 animals per replicate after hatching). - Number of vessels/concentration: in the study report, a number of 28 eggs incubation cups (50 eggs/cup) is indicated and among them two eggs cups have been suspended in each duplicate test aquarium per exposure concentration and the controls; this suggests two vessels per concentration and control (In addition tables results in the study report show two replicates, A and B). 3.4.5 In the Table A7.4.3.2-5 *Test conditions*: Dissolved oxygen range in test and controls solutions was 9.6-10 mg/L. 3.4.6 Duration of the test : *90 days* (60 days post-hatching) 3.4.8 According to the study report, monitoring of the test parameters is not performed only at the end of test e.g. a definitive determination of eggs viability and calculations of percentage survival organisms at hatch have been made on day 18 and day 31 respectively. 4.1.1 In compliance with the study report, the nominal concentrations of the preliminary testing are: 1.6, 3.1, 6.2, 12 and 25µg/L. 4.1.2 ~~*Survival was reduced at 1226 µg/l and above*~~ Survival of 100, 100, 90, 85 and 85% was recorded in the 1.6, 3.1, 6.2, 12 and 25 µg/L treatment levels respectively.
Results and discussion	Applicant's version is acceptable with the following amendements : 5.2 According to OECD Guidelines 215, the validity criterion on the water

	temperature is not fulfilled (a minimum/maximum temperature range of 10 – 19°C were recorded). In addition, the concentrations of the test substance have not been maintained within + 20% of the mean measured values: it should be indicated in the Table A7.4.3.2-6 that "*results based on the measured concentrations."*
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	The study is considered acceptable as supportive to the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.3 Bioaccumulation in an aquatic organism **Annex Point IIIA, XIII.2.3**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [x]**	
Detailed justification:	In addition to negligible exposure, there are data on the potential for bioaccumulation of fipronil in fish from study R010561, summarized in section 7.4.2.	
Undertaking of intended data submission [ ]		

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 11, 2006
Evaluation of applicant's justification	Agree with applicant's justification: the environmental risk assessment for fipronil shows that there is no risk of secondary poisoning under normal conditions of use in Goliath Gel Initial's insecticide (PT18) products. Therefore it is not considered necessary to submit further testing.
Conclusion	Applicant's justification is acceptable
Remarks	None
	COMMENTS FROM …
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	

Section 7.4.3.4 **Annex Point IIIA, XIII.2.4**	**Effects on reproduction and growth rate with an appropriate invertebrate species**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.3.4/01** XXXX The Chronic Toxicity of M&B 46030 to *Daphnia magna* under Flow-Through Conditions (unpublished) (XXXX)	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline 202	X
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	M&B 46030 (fipronil)	
3.1.1	Lot/Batch number	JJW2092	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	97.2% w/w	X
3.1.4	Composition of product	Technical material	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not recorded	X
3.2	**Preparation of TS solution for poorly voluble or volatile test substances**	See Table A7.4.3.4-1	X
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.3.4-2	
3.4.2	Test organisms	See Table A7.4.3.4-3	X

Section 7.4.3.4 Annex Point IIIA, XIII.2.4		**Effects on reproduction and growth rate with an appropriate invertebrate species**	
3.4.3	Handling of offspring	At each observation interval offspring were removed, counted and discarded	
3.4.4	Test system	See Table A7.4.3.4-4	
3.4.5	Test conditions	See Table A7.4.3.4-5	X
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	Survival, no of offspring and body length	
3.4.8	Examination / Sampling	Adult survival and offspring production were measured on days 1, 2, 4 and thereafter 3 times a week from day 7 to day 21	
3.4.9	Monitoring of TS concentration	Samples taken on days 0, 7, 14 and 21	
3.4.10	Statistics	Dunnett's or Williams tests	
		4. RESULTS	
4.1	**Range finding test**	Yes	
4.1.1	Concentrations	100 – 6.3 µg/l	
4.1.2	Number / Percentage of animals showing adverse effects	After 7 days 70% and 90% survival was observed among organisms exposed to 100 and 50 µg/l fipronil respectively. All surviving daphnids in the 100 µg/l test concentration were pale and small in size compared to the control organisms.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	See Table A7.4.3.4-7	
4.2.2	Actual concentrations of test substance	See Table A7.4.3.4-7	
4.2.3	Effect data	See Table A7.4.3.4-7	
4.2.4	Concentration / response curve	None observed	
4.2.5	Other effects		
4.3	**Results of controls**	See Table A7.4.3.4-7	
4.4	**Test with reference substance**	Not applicable	
4.4.1	Concentrations		
4.4.2	Results		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: fipronil purity 100% Test organism: neonate *Daphnia magna* (≤ 24 hours old at test initiation), fed during the test a mixture of green algae, trout food and a commercial mixture of proteins and fatty acids. Dilution water: fortified well water (hardness 170 mg/l as $CaC0_3$), temperature 20± 1°C, oxygen content ≥ 7.0mg/l, pH 7.7 – 8.4.	X

Section 7.4.3.4 Annex Point IIIA, XIII.2.4		**Effects on reproduction and growth rate with an appropriate invertebrate species**	
		Exposure regime: flow through exposure over 21 days. Nominal test item concentrations based on the result of preliminary testing: 0 (control), 0 (solvent control, acetone at 17µg/l), 6.3, 13, 25, 50 and 100 µg/l.	
		Each concentration consisted of 4 replicate vessels with 10 daphnids each. Adult survival was recorded in days 1,2, 4 and survival and offspring production were recorded three times a week from day 7 to day 21. At test termination, the body length of all surviving daphnids was measured. Samples for chemical analysis were taken from all exposure concentrations of days 0, 7, 14 and 21. The 21 day NOEC was determined based on Dunnett's or Williams tests.	
5.2	**Results and discussion**	The actual mean measured concentrations were 5.0, 9.8, 20, 34 and 79 µg/l (an average of 74% of the nominal concentrations).	X
		Unforeseen mortality occurred in the control group but did not occur in the solvent control. The effect concentrations were determined in comparison to the solvent control.	X
		Significant mortality was observed at 34 and 79 µg/l. No significantly different survival rate was observed at the remaining concentrations. No effect on the reproductive performance was observed at concentrations below the lethal levels.	
		First offspring was recorded at 5.0, 9.8, and 20µg/l on day 10 and on day 12 at 34µg/l, in the control and in the solvent control. The mean length of daphnids exposed at 20µg/l was significantly different form the solvent control, however, there was no difference in the reproductive performance at this concentration. Based on mean body length, the 21-day NOEC was reported at 9.8 µg/l. However, survival and reproduction are presumably the most relevant ecological key parameter. Based on these parameters, the 21-day NOEC would be determined at 20µg/l.	
5.2.1	NOEC	9.8µg/l based on mean body length. 20µg/l based of survival and reproductive performance	
5.2.2	LOEC	20 µg/l based on mean body length.	
5.2.3	EC_{50} (EC_x)	Not determined	X
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Table A7.4.3.4-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	
Vehicle	Acetone
Concentration of vehicle	5.9mg/ml
Vehicle control performed	Yes
Other procedures	No

Table A7.4.3.4-2 Dilution water

Criteria	Details
Source	Well water fortified according to the formula for hard water (ASTM 1960) and filtering to remove any potential organic contaminants
Alkalinity	110 – 130mg/l ($CaC0_{3)}$
Hardness	160 – 180mg/l ($CaC0_{3)}$
pH	7.9 – 8.3
Ca / Mg ratio	Not recorded
Na / K ratio	Not recorded
Oxygen content	7.4 – 8.0 mg/l
Conductance	400 – 600 microhomos
TOC	Not recorded
Holding water different from dilution water	No identical to holding and culturing water

Table A7.4.3.4-3 Test organisms

Criteria	Details
Species / Clone	*Daphnia magna*
Source	XXXX
Age	< 24 hours
Breeding method	Not reported
Kind of food	Green algae and a trout food suspension
Amount of food	Not recorded
Feeding frequency	Daily
Pre-treatment	None
Feeding of animals during test	Yes – as in culture

Table A7.4.3.4-4 Test system

Criteria	Details
Test type	Flow through
Renewal of test solution	6 aquarium volumes per 24 hours
Volume of test vessels	1.4 litres
Volume/animal	140ml
Number of animals/vessel	10
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.3.4-5 Test conditions

Criteria	Details
Test temperature	20 ± 2°C
Dissolved oxygen	7.4 – 8.0 mg/l
PH	7.9 – 8.3
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	40 – 90 foot-candles
Photoperiod	16 hours light/8 hours dark

Table A7.4.3.4-6 Validity Criteria for Invertebrate Reproduction Test According to OECD Guideline 211

	Fulfilled	Not fulfilled
Mortality of parent animals < 20% at test termination	✓	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	✓	
Criteria for poorly soluble test substances	✓	

Table A7.4.3.4-7 Table of effects

Exposure concentrations		21-day results (mean and SD)		
Nominal µg/l	**Mean Measured µg/l**	**Survival (%)**	**Offspring (#/parent)**	**Body length (mm)**
0 (control)	< LOQ	50 ± 18	121 ± 36	4.4 ± 0.21
0 (solvent control)	< LOQ	98 ± 5	100 ± 8	4.7 ± 0.15
6.3	5.0	90 ± 8	108 ± 31	4.8 ± 0.24
13	9.8	90 ± 14	132 ± 31	4.8 ± 0.16
25	20	95 ± 6	101 ± 9	4.4 ± 0.20*
50	34	63 ± 22*	27 ± 19(*)	4.9 ± 0.28(*)
100	79	0 ± 0*	0 ± 0(*)	-

(*) due to a significant survival effect at this level, sublethal data were not statistically analysed.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 11, 2006
Materials and methods	The applicant's version is adopted with the following revisions: 1.1 Reference : *McNamara, P.C.* ~~*(1995)*~~ (1990) 2.1 The test was conducted from December 1989 according to OECD Guideline 202, Part II, *Daphnia* sp Reproduction Test (adopted April 1984). It should be noted that the recommended study guideline by TNsG on Data Requirements is OECD Guideline 211 (adopted September 1998) ant that chronic data from tests performed according to Guideline 202 could be variable. 3.1.3 Purity : ~~*97.2%*~~ 100% 3.1.6 A gas chromatographic procedure is indicated in the study report to analysis of test item. 3.2 In the *Table A7.4.3.4-1:* ▪ In compliance with the study report, 5.9 mg/mL is the concentration of stock solution and not the one of acetone. However, it could be indicated that "the solvent control contained the greatest amount of acetone present in any test concentration i.e. 17µL/L". 3.4.3. In *Table A7.4.3.4-3 Test organisms*: ▪ In compliance with the study report, the age of Daphnis is ≤ 24 hours and not < 24 hours. ▪ Detailed information on feeding are given in the study report such as: 1) the diet consisting of a suspension of trout food (5mg/mL), green algae (4×10^7 cells/mL) and Selco (0.6 mg/mL); 2) the rate of diet is of 2 mL, 3 mL and 0.5 mL of trout food, algae suspension and selco respectively; 3) the frequency of feeding is of three times daily on weekdays and twice daily on weekends. 3.4.5 In the Table A7.4.3.4-5 *Test conditions*: ▪ Dissolved oxygen range in test and controls solutions was 7 – 8.4 mg/L and pH range was 7.7 – 8.4 5.1 *Dilution water: fortified well water (hardness:* ~~*170*~~ 160 – 180 *mg/l as* $CaC0_3$*), temperature 20± 1°C, pH* ~~*7.7 – 8.4*~~ 7.9 – 8.3.
Results and discussion	Applicant does not discuss the results and principally two points: 1) The unexpected decrease in the survival of the dilution water control daphnids. 2) According to OECD Guideline 211, the reproductive output of *Daphnia magna* is estimated from the total number of living offspring produced per parent animal alive at the end of the test. This means that juveniles produced by adults that die during the test are excluded from the calculations. As there is no

	indication in this study on this procedure, the reproductive output of *Daphnia magna* could have been overestimated by counting all offspring (juveniles produced by adults alive and die) and lead to an underestimation of the NOEC. 5.2 Some points need revisions/amendments: ▪ In compliance with the study report, the mean measured concentrations of test material averaged 76 % (and not 74 %) of the nominal concentrations with a minimum of 68%. ▪ According to OECD Guidelines 211, the validity criterion on the mortality of parent animals in the controls is not fulfilled because it is only fulfilled for the solvent control. In addition, it should be noted that the coefficient of variation around the mean number of offspring produced per parent animal in the control is > 25% and in accordance with the OECD Guideline 211 it should be ≤ 25 % in a well-run test. ▪ In addition, the concentrations of the test substance have not been maintained within 20% of the mean measured values: it should be indicated in the Table A7.4.3.4-6 that "*results are based on the measured concentrations."* 5.2.3 An EC50 of 39 µg/L has been estimated in the study report.
Conclusion	Applicant does not make any conclusions. 5.3.2 there is a major deficiency (see above comments on the mortality of parents animal in the control). However, as all the statistical comparisons to determine treatment level effects have been performed using the solvent control data, it is considered that this deficiency did not affect the applicability of the results.
Reliability	The reliability should be set to 2.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.4 **Annex Point IIIA, XIII.2.4**	**Effects on reproduction and growth rate with an appropriate invertebrate species**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.3.4/02** XXXX Effect of ^{14}C Fipronil on the Development of Sediment Dwelling Larvae of *Chironomus riparius* in a Water Sediment System 30 September 2004 (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Draft Document February 2001, Proposal for a new guideline 219 "Sediment-Water Chironomid Toxicity Test Using Spiked Water	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	^{14}C fipronil	
3.1.1	Lot/Batch number	NXG 3027/2	
3.1.2	Specification	Not given	
3.1.3	Purity	99.14%	
3.1.4	Further relevant properties	None	
3.1.5	Radiolabelling	Yes	
3.1.6	Method of analysis	HPLC	X
3.2	**Reference substance**	None	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing/estimation procedure**		
3.3.1	Test system / performance	See Tables Table A7.4.3.4-9, 10, 11 and 12	X

Section 7.4.3.4 **Annex Point IIIA, XIII.2.4**	**Effects on reproduction and growth rate with an appropriate invertebrate species**

3.4	**Test Organisms**	See Table A7.4.3.4-8	
		4. RESULTS	X
4.1	**Experimental data**	See Table A7.4.3.4-13	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The effect of ^{14}C Fipronil on the development of sediment dwelling larvae of the midge Chironomus riparius was investigated according to OECD draft Guideline 219. A water sediment system was used in this long term chronic study over 28 days in which the test compound was applied to the water phase. The following concentrations of fipronil were tested: 10,20, 40, 80, 160 and 320 ng/l (nominally), and a solvent control each with four replicates plus a solvent free control with six replicates	X
5.2	**Results and discussion**	Analytical results Total radioactivity measurements of test item ^{14}C fipronil were performed in overlying water, pore water and sediment (in pore water and sediment only at the highest test item concentration). The concentration of total applied radioactivity in the water samples at DAT 0 ranged from 98.0% to 104% of the nominal concentration. However the HPLC analysis of the test item in the stock solution yielded a content of 73%. Therefore, taking the measured content of the ^{14}C fipronil into consideration the following initial concentrations were yielded 0, 7.3, 14.6, 29.2 58.4, 116.8 and 233ng/l. The results are based on HPLC measurement corrected values. Sediment concentrations in the highest treatment group increased from 226.0 ng/kg (dry wt) at DAT 2 to 510.4 ng/kg at test termination. At DAT 7 sediment concentrations were found to be 385 ng/kg Thus chironomid larvae were exposed to both water and sediment residues. Biological results Statistically significant effects in the emergence and development rates were obtained at the highest test concentration of 233.6 ng/l only. At this level, emergence was nearly completely reduced (1 midge emerged during the observation period). No adverse effects were observed at lower concentrations. Therefore, the NOEC found in this study was 117 ng/l based on initial measured fipronil concentrations in water. Based on concentrations in sediment at the highest concentration tested a NOEC of 193 ng/kg dry wt was estimated.	X X
5.2.1	NOEC	117 ng/l	X
5.2.2.	LOEC	234 ng/l	X
5.3	**Conclusion**		X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	

Table A7.4.3.4-8 Test organisms

Criteria	Details
Species / Clone	*Chironomus riparius*
Source	XXXX
Age	< 3 days
Breeding method	Not recorded
Kind of food	Algae and commercially available fish feed
Amount of food	Not recorded
Feeding frequency	Regularly, ad libitum
Pre-treatment	None
Feeding of animals during test	Commercially available fish feed 0.25 – 1 mg per larva per day up to day 27

Table A7.4.3.4-9 Dilution water

Criteria	Details
Source	Synthetc Medium (M4 according to Elendt)
Alkalinity	0.89 mmol/l
Hardness	2.40 mmol/l
pH	7.97
Conductance	650 µS/cm

Table A7.4.3.4-10 Artificial Sediment

Criteria	Details
Source	Prepared as described in OECD Guideline 219
Composition	5% Sphagnum peat 20% Kaolin (kaolinite content ≥ 30%) 0.5% quartz sand particle size ≥ 32% 0.063 -0.2mm
pH	7.24

Table A7.4.3.4-11 Test system

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	600ml
Volume/animal	21ml
Number of animals/vessel	10
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.3.4-12 Test conditions

Criteria	Details
Test temperature	20 ± 2°C
Dissolved oxygen	7.4 – 8.0 mg/l
PH	7.9 – 8.3
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	40 – 90 foot-candles
Photoperiod	16 hours light/8 hours dark

X

Table A7.4.3.4-13 Table of effects

Initial Water Concentration (nominal) ng/l	Initial Water Concentration (measured) ng/l	Midge emergence		Midge development	
		Emergence rate	% reduction	Development rate	% reduction
Control	Control	0.992	-5.8	0.0693	3.5
Solvent control	Solvent control	0.938	0.0	0.0718	0.0
10	7.3	0.988	-5.3	0.0705	1.8
20	14.6	0.913	2.7	0.0695	3.2
40	29.2	0.875	6.7	0.0705	1.8
80	58.4	0.950	-1.3	0.0695	3.2
160	116.8	0.875	6.7	0.0678	5.6
320	233.6	0.013*	98.6*	0.0143*	80.1*

* Statistically different from the control Bonferroni-,Dunnett's-and Williams-test

Table A7.4.3.4-14 Validity Criteria for Sediment-Water Chironomid Toxicity Test Using Spiked Water following OECD Guideline 219

	Fulfilled	Not fulfilled
Emergence in controls at least 70% at test end	✓	
Emergence in controls between day 12 and 23 after insertion of the larvae	✓	
Oxygen in all vessels at least 60% of ASV	✓	
pH of overlying water in all vessels between 6 and 9	✓	
Water temperature should not differ by more than ± 1.0°C	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 12, 2006
Materials and methods	The applicant's version is adopted with the following revisions/amendments: 3.1.6 The radioactivity was measured with LSC (liquid scintillation counting) and radio-HPLC (high performance liquid chromatography). 3.3.1 In the Table A7.4.3.4-11 *Test system:* ▪ Number of animals/vessel: *20 Chironomus riparius* larvae added to each vessel. In the Table A7.4.3.4-12 *Test conditions:* ▪ Temperature: *19 – 20.6 °C* ▪ Dissolved oxygen: *6 – 10 mg/L* ▪ PH: *7.16 – 8.41* Subsection 3 needs more information such as: 3.5 Test parameters : emergence and development rates. 3.6 Sampling/examination : Visual assessments (behavioural, mortalities, emergence) were made at least on each working day. The number, time and sex of emerged adults was recorded. 3.7 Monitoring of TS : the concentration of radioactivity in water samples has been measured by LSC at DAT 0 after the application. The concencentration over time has been measured in water, pore water and sediment in the vessels treated with the highest rate at DAT 0, 2, 7, 14 and 28. 3.9 Statistics : ANOVA, Dunnett's, Bonferroni's and Williams' test for emergence and development rate (a = 0.05), log-log and logit analysis for determination of the EC50 values.
Results and discussion	The applicant's version is adopted with the following revisions/amendments: *Total radioactivity measurements of test item ^{14}C fipronil were performed* at DAT 0 *in overlying water, pore water and sediment (in pore water and sediment only at the highest test item concentration).* ~~*However the HPLC analysis of the test item in the stock solution yielded a content of 73%*~~ However the HPLC analysis of the test item in the stock solution yielded a content of ^{14}C Fipronil 73% of the total radioactivity According to the HPLC measurements, a correction for the chemical purity of the batch was made and biological results are based on these values. Total radioactive residues (TRR) in the overlying water were reduced by 73% of the initial activity at the end of the study; simultaneously, test item concentrations in sediment increased from 20.3% TRR at DAT (day after treatment) 2 to 41.7% TRR at DAT 28 in the highest treatment level. *Sediment concentrations in the highest treatment group increased from* 0.226 µg/kg dry

wt (based on initial measured ^{14}C-fipronil concentrations) *at DAT 2* to 0.5104 µg/kg dry wt *at test termination.* At DAT 7, sediment concentrations were found to be 0.3854 µg/kg dry wt. Pore water concentrations were on average 0.05 µg/L and did not differ much during the course of the study. *Thus chironomid larvae were exposed to both water and sediment residues.*

Results based on initial measured concentrations :

5.2.1 NOEC :	Emergence rate : 116.8 ng/L	
	Development rate : 116.8 ng/L	
5.2.2 LOEC:	Emergence rate : 233.6 ng/L	
	Development rate : 233.6 ng/L	
5.2.3 EC_{50}:	Emergence rate : 144.8 ng/L	
	Development rate : 188.99 ng/L	

Based on sediment residues of the highest test concentration at DAT 7 (which are considered as the mean concentration), the following NOEC for the respective emergence and development rate was calculated : NOEC = 192.5 ng/kg (sediment).

Remarks: The method to estimate a NOEC for sediment should be detailed and discussed:
Since no chemical analysis of the sediment was available in the no-effect level treatment group (i.e. at 0.117 µg/L), it has been assumed in the study that sediment concentrations are proportional to the initially measured water concentrations and the sediment analysis of DAT 7 at 0.234 µg/L (measured conc.) was used, which yielded 0.3854 µg/kg dry sediment (after correcting for chemical purity of 73%). Hence, the $NOEC_{sediment}$ was calculated as: 0.385 *(0.117/0.234) = 0.193 µg/kg dry sediment.
According to OECD guideline 219, it can be noted that samples of the overlying water, the pore water and the sediment would have to be also analyzed at a lower concentration for better evaluate the behaviour/partitioning of the tested chemical in the water-sediment system and thus the relation of proportionality between sediment concentrations and water concentrations.

Applicant does not discuss the results. No deficiencies reported.

According to OECD Guidelines 219, the validity criterion on the water temperature is not fulfilled (temperature was differed by more than ± 1.0°C). However it is considered that this deficiency did not affect the applicability of the results.

Conclusion	Applicant does not make any conclusions, particularly on the relevance of the sediment NOEC estimated by extrapolation of results obtained for the water compartment. The study shows that exposure from the sediment compartment is of concern. In addition, given the partitioning and rapid degradability of the substance and as the water NOEC is based on initial measured fipronil concentrations in water, it should be noted that this NOEC take all potential routes of exposure into account but the used methodology does not let to determine whether it is the substance or the degradation/reaction products that have been tested. RMS considers that no valid interpretation can be made, however others member states considered this methodology as acceptable.
Reliability	As the methodology used to determine the toxicity endpoints is likely not appropriate to the unstable chemical, the reliability should be set to 2.
Acceptability	As no valid interpretation can be made, the study should normally not be considered acceptable as supportive data to the risk assessment of the water compartment. In addition, the test design does not fulfil the requirements of the directive to determinate a realistic risk assessment of the sediment compartment (only the test using spiked sediments is considered appropriate). However, as *C.riparius* seems to be the most sensitive specie of fresh water systems, this study is considered acceptable as supportive data to the risk assessment of the water and sediment compartments.
Remarks	

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.4.3.4/03 NON KEY STUDY** XXXX Fipronil - Chronic toxicity to mysids (*Mysidopsis bahia*) under flow-through conditions. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes FIFRA Guideline 72-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	[^{14}C] MB46030 (= radiolabeled Fipronil)	
3.1.1	Lot/Batch number	Lot GHS 771A	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	97.7%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	Liquid scintillation counting procedure (LSC)	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_3_4-1	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	

Section 7.4.3.4 Annex Point IIIA XIII.2.4		**Effects on reproduction and growth rate with an invertebrate species**	
3.4.2	Test organisms	Juvenile mysids (*Mysidopsis bahia*), ≤ 24 h of age (current name: *Americamysis bahia*) See also table A7_4_3_4-3	
3.4.3	Handling of offspring	Not reported	
3.4.4	Test system	Exposure regime: 28-day flow-through. Test groups consisted of two replicate aquaria with 30 mysids each (in two retention chambers with 15 mysids each) per concentration. See table A7_4_3_4-4	
3.4.5	Test conditions	See table A7_4_3_4-5	
3.4.6	Duration of the test	28 days	
3.4.7	Test parameter	Abnormal behaviour or appearance Body length and dry weight Reproductive success	
3.4.8	Examination / Sampling	Abnormal behaviour and appearance of the mysids was monitored daily. Total body length and dry weight of all surviving mysids was determined after 28 days. Reproductive success was based on offspring produced per female and day. Exposure concentrations were sampled for radiochemical analysis once weekly. Identity and stability of the test item were confirmed chromatographically in the highest concentration.	
3.4.9	Monitoring of TS concentration	Exposure concentrations were analytically confirmed on days 0, 7, 14, 21, and 28	
3.4.10	Statistics	Significance of effects of exposure to the test item was judged based on Williams' test, compared to pooled control, coupled with Bartlett's test for determination of homogeneity of variances.	
		4. RESULTS	
4.1	**Limit test / Range finding test**	A preliminary 20-day exposure was conducted with mysids (≤ 24 h old) exposed to various fipronil concentrations (one replicate per treatment level) under static conditions with daily solution renewals.	
4.1.1	Concentrations	3.1, 6.2, 12, 25 and 50 ng a.s./L, a control and a solvent control	
4.1.2	Number/ percentage of animals showing adverse effects	Not reported	
4.1.3	Nature of adverse effects	Not reported	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	4.4, 8.8, 18, 35 and 70 ng a.s./L (nominal)	
4.2.2	Actual concentrations of test substance	5.0, 7.7, 15, 28 and 57 ng a.s./L The mean measured concentrations ranged 81-114% of nominal.	
4.2.3	Effect data	See table A7_4_3_4-6	

Section 7.4.3.4 Annex Point IIIA XIII.2.4		**Effects on reproduction and growth rate with an invertebrate species**	
4.2.4	Concentration / response curve	Not reported	
4.2.5	Other effects	-	
4.3	**Results of controls**	See table A7_4_3_4-6	
4.4	**Test with reference substance**	No	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil was tested in a 28-day life-cycle toxicity test with *Mysidopsis bahia* (≤ 24 hours old) under flow-through conditions according to the requirements of FIFRA Guideline 72-4. In the study, the nominal concentrations 4.4, 8.8, 18, 35 and 70 ng a.s./L (mean measured concentrations 5.0, 7.7, 15, 28 and 57 ng/L) were tested and compared to a solvent and dilution water control. Tested endpoints were survival, behaviour, reproduction success and growth (total body length and dry weight) of the mysids. At maturity of the mysids (day 15 of exposure), 10 pairs were transferred into pairing chambers. The remaining mysids were maintained until the end of the chronic test and served to replace any dead males in the pairing chambers, if necessary. Dead females were not replaced. After males and females had been paired, the number of dead males and females, the number of offspring produced by each individual female, and any abnormal appearance or behaviour was recorded daily. Dead parental mysids and offspring were recorded, removed, and discarded when observed during the test. The statistical method to be used to evaluate the results was the Williams' Test, coupled with Bartlett's test for determination of homogeneity of variances.	
5.2	**Results and discussion**	Survival of both males and females, length and dry weight of females and reproductive success were statistically comparable to the pooled control up to and including 28 ng a.s./L. Male length was significantly different at and above 15 ng a.s./L, and male dry weight was significantly different at and above 5.0 ng a.s./L. Based on these comparisons, male dry weight was identified as the statistically most sensitive parameter. However, the difference in the male body-weight was 10-16% compared to pooled control, without dose-response throughout a concentration range spanning a factor of 10X. For this reason, the difference in the male body weight was not considered as indicator of toxicity of Fipronil to mysid shrimps. The LOEC for Fipronil and mysid shrimp was based on effects on male length and determined at 15 ng a.s./L and above, since there was a clear and consistent dose-response observed in this parameter. Consequently, the overall NOEC was determined at 7.7 ng a.s./L. See also table A7_4_3_4-6	

Section 7.4.3.4 Annex Point IIIA XIII.2.4	**Effects on reproduction and growth rate with an invertebrate species**	
5.2.1 NOEC	7.7 ng a.s./L	
5.2.2. LOEC	15 ng a.s./L	
5.2.3 EC_{50} (EC_x)	-	
5.3 Conclusion	The 28-d NOEC of Fipronil in the mysid shrimp, *Mysidopsis bahia*, was determined at 7.7 ng a.s./L (based on mean measured concentrations). The test is considered valid as: - less than 30% of the first generation control mysids died between paring and the end of the test - more than 75% of the paired first generation females in the controls produced young or the average number of young produced by the paired first generation females in the controls is more than three	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Table A7_4_3_4-1: Preparation of TS Solution for Poorly Soluble or Volatile Test Substances

Criteria	**Details**
Dispersion	-
Vehicle	Acetone
Concentration of vehicle	≤ 0.1 mL/L
Vehicle control performed	Yes A 0.52 mL/mL solvent control stock solution was prepared by diluting 52 mL of acetone with distilled water to a volume of 100 mL.
Other procedures	The stock solution was prepared by solving the entire amount of [^{14}C]fipronil received (0.46 mCi) with acetone to a volume of 50 mL. The stock solution concentration was determined to be 0.214 mg/mL, based on the analysis and the specific activity of 18.9 mCi/mmole (9.5838 x 10^4 dpm/µg).

Table A7_4_3_4-2: Dilution Water

Criteria	Details
Source	Artificial seawater
Salinity	25 ± 3‰
Hardness	20 – 40 mg/L as $CaCO_3$
pH	7.7 – 8.3
Ca / Mg ratio	Not given
Na / K ratio	Not given
Oxygen content	Not given
Conductance	Not given
TOC	0.49 mg/L
Holding water different from dilution water	No

Table A7_4_3_4-3: Test Organisms

Criteria	Details
Strain / Clone	*Mysidopsis bahia* (current name: *Americamysis bahia*)
Source	Springborn Laboratories cultures (SLI Lot #95A24) The brood stock was originally obtained from XXXX
Age	≤ 24 hours old
Breeding method	Mysids were cultured in 76 L glass aquaria with a closed-loop recirculating filtration system providing artificial seawater to the aquaria. Salinity: 26 - 27‰ pH: 8.2-8.3 Photoperiod: 16 h light: 8 h dark Temperature: 24 - 25 °C
Kind of food (breed)	Live brine shrimp (*Artemia salina*) nauplii (≤ 48 h old) Selco®, a supplemental substance high in saturated fatty acids
Amount of food (breed)	*Ad libitum*
Feeding frequency (breed)	Twice daily with shrimp; one feeding supplemented with Selco®
Pre-treatment	Not reported
Feeding of animals during test	Mysids were fed live brine shrimp nauplii, twice daily. During the period prior to pairing, at least one of these feedings was with brine shrimp nauplii enriched with Selco®. Following the pairing phase, the mysids were fed brine shrimp nauplii enriched with Selco® on an every-other-day basis.

Table A7_4_3_4-4: Test System

Criteria	Details
Test type	28 days chronic toxicity to mysids under flow-through conditions Exposure regime: 28-day flow-through. Test groups consisted of two replicate aquaria with 30 mysids each (in two retention chambers with 15 mysids each) per concentration. At maturity of the mysids (day 15 of exposure), 10 pairs were transferred into pairing chambers. The remaining mysids were maintained until the end of the chronic test and served to replace any dead males in the pairing chambers, if necessary. Dead females were not replaced. Retention chambers consisted of approximately 10 cm diameter by 2 cm high glass Petri dishes to which a 15 cm high, 363 µm Nitex® nylon screen collar was attached with clear silicone adhesive. Pairing chamber were cylindrical glass jars (5.1 cm diameter x 10 cm depth) in which two 2 cm holes were drilled and covered with 363 µm Nitex® screens. A maximum of ten pairing chambers were impartially placed in each test chamber and appropriately labelled.
Endpoints	Abnormal behaviour or appearance of the mysids were monitored daily. Total body length and dry weight of all surviving mysids was determined after 28 days. Reproductive success was based on offspring produced per female and day.
Sediment	-
Renewal of test solution	Yes Flow-through system
Volume of test vessels	19.5 L all-glass aquaria (39 x 20 x 25 cm)
Volume/animal	19.5 L / 30 mysids
Number of animals/vessel	30 mysids each (in two retention chambers with 15 mysids each)
Number of vessels/concentration	2 replicates per treatment level
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test Conditions

Criteria	Details
Test temperature	~~26 ± 27 °C~~ **26 – 27°C**
Salinity	~~26 – 27‰~~ **25 – 27‰**
Dissolved oxygen	86 – 104% of saturation throughout the test period
pH	8.1 – 8.3
Adjustment of pH	-
Aeration of dilution water	-
Quality/Intensity of irradiation	220-810 lux
Photoperiod	16 h light: 8 h dark
Control of ammonia levels	Not reported

Table A7_4_3_4-6: Summary of data on the chronic toxicity of fipronil to mysids (*Mysidopsis bahia*) under flow-through conditions

Nominal concentr. [ng a.s./L]	Mean measured [ng a.s./L]	28d survival (%)	Reprod. success	Length, males (mm, ± SD)	Length, females (mm, ± SD)	Dry Wt., males (mg, ± SD)	Dry Wt., females (~~mm~~ mg, ± SD)
Control	< QD	85	0.46	7.4 ± 0.4	7.0 ± 0.3	0.84 ± 0.11	0.93 ± 0.16
Solv. control	< QD	85	0.24	7.2 ± 0.3	7.1 ± 0.3	0.78 ± 0.08	0.94 ± 0.19
Pld. control	< QD	85	0.35	7.3 ± 0.4	7.0 ± 0.3	0.81 ± 0.10	0.93 ± 0.17
4.4	5.0	82	0.36	7.0 ± 0.7	7.0 ± 0.4	0.70 ± 0.14 *	0.92 ± 0.19
8.8	7.7	83	0.32	7.2 ± 0.3	7.1 ± 0.4	0.73 ± 0.09 *	0.86 ± 0.19
18	15	88	0.24	6.9 ± 0.5 *	6.9 ± 0.4	0.68 ± 0.11 *	0.85 ±0.19
35	28	72	0.16	6.8 ± 0.5 *	6.8 ± 0.4 *	0.69 ± 0.11 *	0.89 ± 0.17
70	57	53 *	0.029 (*)	6.6 ± 0.3 (*)	6.7 ± 0.5 (*)	0.73 ± 0.11 (*)	0.82 ± 0.15 (*)

***: significantly different from the pooled control, based on Williams' Test.**
(*): due to a significant effect on survival at this level, sublethal data were not statistically analysed.
Pld. control: Pooled control, since control and solvent control data were not determined to be significantly different (t-Test), all treatment data were compared to the growth of the pooled control organisms.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13 November 2007
Materials and methods	Agree with the applicant's version. Errors in Table A7.4.3.4-5 are corrected in bold and underlined. Additional information are added in bold and underlined in Table A7.4.3.4-6
Conclusion	Agree with the applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	The non-key study status will be discussed with the other RMS.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.4.3.4/04 NON KEY STUDY** XXXX Fipronil – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Under Static Conditions in a Water-Sediment System. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OPPTS Draft Guideline Number 850.1350: Mysid Chronic Toxicity Test (U.S. EPA, 1996) U.S. EPA's Pesticide Assessment Guidelines: Subdivision E (U.S. EPA, 1982) Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids (ASTM, 1994)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Exceptions: routine water, sediment and food contaminant screening analyses were conducted at GeoLabs, Inc., Braintree, MA, USA, using standard U.S. EPA procedures and are considered facility records under Springborn Smithers Laboratories' SOP 7.92. Since the analyses were conducted following standard validation methods, this exception has no impact on the study.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil (AE F124964 00 1B99 0002)	
3.1.1	Lot/Batch number	Batch No. BES1905	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	99.7%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	

Section 7.4.3.4 Annex Point IIIA XIII.2.4		**Effects on reproduction and growth rate with an invertebrate species**	
3.1.6	Method of analysis	LC/MS/MS procedure based on a methodology validated at Springborn Smithers. The method validation studies were conducted prior to the initiation of the test and established an average recovery of 95.6 ± 3.45% for fipronil from freshwater and 72.8 ± 4.02% from sediment. A method verification with filtered seawater indicated that appropriate recoveries were obtained for fipronil in seawater.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_3_4-1	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	*Americamysis bahia* (sexually mature, 21 days old) and *Americamysis bahia* (≤ 24 hours old) See also table A7_4_3_4-3	
3.4.3	Handling of offspring	Not reported	
3.4.4	Test system	28-day life-cycle toxicity test under static conditions See table A7_4_3_4-4	
3.4.5	Test conditions	See table A7_4_3_4-5	
3.4.6	Duration of the test	28 days	
3.4.7	Test parameter	Group 1: (parental mysids, 21 days old) exposed from day 0 to day 14: survival and reproduction Group 2: (≤ 24 hours old mysids) exposed from day 0 to day 28: survival, reproduction, growth (length and weight) and free-ranging population (number of adults, number of juveniles)	
3.4.8	Examination / Sampling	Group 1 & 2: Survival of paired mysids and number of offspring per female was determined daily Group 2: Additionally, total lengths and dry weights were determined of all surviving first generation mysids held only in the pairing chambers at test termination Free-ranging mysids: The number of juvenile and mature mysids was counted at test termination	
3.4.9	Monitoring of TS concentration	On day 0, 4, and weekly, thereafter	X

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**	
3.4.10 Statistics	All statistical conclusions were made at the 95% level of certainty except in the cases of Bartlett's Test and Shapiro-Wilk's Test, in which the 99% level of certainty was applied. The following procedures were used: 1) Significant differences in the percentage survival were determined 2) As a check on the assumption of homogeneity of variance implicit in parametric statistics, data for each endpoint were analyzed using Bartlett's Test (Horning and Weber, 1985) 3) Shapiro-Wilk's Test for normality (Weber et al., 1989) was conducted and compared the observed sample distribution with normal distribution. The assumption that observations are normally distributed must be validated before subsequent analyses, following parametric procedures, can be performed. If the data are not normally distributed, then a non-parametric procedure is used for subsequent analyses. 4) Organism survival, reproduction and growth at each treatment level of fipronil was compared with the performance of the solvent control data using Williams' Test (Williams, 1971, 1972) and Bonferroni's T-test (Weber et al., 1989) A computer program (West, Inc. and Gulley, 1996) was used to perform the statistical computations.	
	4. RESULTS	
4.1 Limit test / Range finding test	Not performed	
4.1.1 Concentrations	-	
4.1.2 Number/ percentage of animals showing adverse effects	-	
4.1.3 Nature of adverse effects	-	
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	The nominal initial concentrations of the test substance were: 15, 30, 60 ng a.s./L.	

Section 7.4.3.4 Annex Point IIIA XIII.2.4		**Effects on reproduction and growth rate with an invertebrate species**	
4.2.2	Actual concentrations of test substance	Analytical verification of test item concentrations in the overlying water was conducted in each concentration. Mean recoveries of Fipronil were in the range of 91.7% - 113.3% of nominal concentrations at test initiation. On test day 4, measured concentrations decreased to 20.0% - 42.7%. By test day 28 (test termination), measured concentrations decreased to below detection levels (i.e., 0.004 µg a.s./L). Measurement of solvent control samples showed contamination in one replicate, which was therefore excluded from for the evaluation of the results. Sediment concentrations were determined in the high treatment level. As expected, concentrations were below LoQ at test initiation. On test day 4, 7, 14, 21 and 28, measured concentrations of Fipronil in sediment were 0.140, 0.115, 0.078, 0.098 and <0.030 µg a.s./kg, respectively. Based on the analytical results of overlying water and sediment in this study, Fipronil concentrations in the overlying water decreased throughout the exposure period with at least some of the Fipronil partitioning to the sediment. As the measurements confirmed the correct application of the test substance (nominal initial concentrations were 91.7-113.3% of nominal), the following biological results were based on nominal concentrations.	
4.2.3	Effect data	See table A7_4_3_4-6 to table A7_4_3_4-8	
4.2.4	Concentration / response curve	-	
4.2.5	Other effects	-	
4.3	**Results of controls**	See table A7_4_3_4-6 to table A7_4_3_4-8	
4.4	**Test with reference substance**	No reference substance used.	
4.4.1	Concentrations	-	
4.4.2	Results	-	

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**	
.1 Materials and methods	Fipronil was tested in a 28-day life-cycle toxicity test with *Americamysis bahia* (≤ 24 hours old and sexually mature, 21 days old) under static conditions. In the study, the nominal concentrations 15, 30, 60 ng a.s./L were tested and compared to a solvent control. Tested endpoints were survival and reproduction of sexually mature mysids and survival, reproduction, growth (length and weight) and free-ranging population (number of adults, number of juveniles) of ≤ 24 hours old mysids. The methods and biological endpoints are based on the requirements of EPA's Ecological Effects Test Guideline OPPTS Draft Guideline Number 850.1350: Mysid Chronic Toxicity Test (U.S. EPA, 1996), U.S. EPA's Pesticide Assessment Guidelines: Subdivision E (U.S. EPA, 1982) and the Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids (ASTM, 1994) as closely as possible. 200 mysids (≤ 24 h old) per test concentration were released in aquariums (4 replicates per treatment group, 50 individuals per aquarium). In addition, 40 sexually mature mysids (20 pairs of 21-day old mysids) were transferred in 20 pairing chambers also placed in the exposure aquariums (five per aquarium) of each treatment level and the solvent control. At test initiation, 21-day old mature male/female pairs were transferred into each of five individual glass pairing chambers. These organisms were used to evaluate mysid reproduction during the initial 14-day exposure (designated group 1). As the aim of this study was the determination of female reproduction, males that died during the exposure period were replaced, whereas dead females were not replaced. Live young in each pairing chamber were counted, recorded and removed daily up until test day 14. At test day 14, all mysids were removed and counted from the retention chambers in order to initiate the mysids pairing of group 2. Survival data for group 1 was based on the number of 21-day old female mysids initially present in each test vessel (i.e., five). For the juvenile mysids (i.e., free-ranging, group 2) exposed at test initiation, the length of time for brood appearance was noted for all first generation mysids prior to pairing (approximately day 10). When first generation mysids reached sexual maturity (approximately day 14), they were redistributed within the same test vessel. Mature male/female pairs were transferred from the aquarium into each of five individual glass retention chambers. The remaining mysids were counted and returned to the aquarium, and remained free-ranging during the remainder of the test. As one of the main objectives of the chronic test was to determine female reproduction, males from this pool were used to replace males from the male/female pairs that died during the test. Females that died were not replaced. Live young in each pairing chamber were counted, recorded, and removed daily until test termination (day 28). Survival was based on the number of 14-day old female mysids initially present in each test vessel (i.e., five). Total lengths and dry weights were determined on all surviving first generation mysids held only in the retention chambers at test termination.	X

Section 7.4.3.4 **Annex Point IIIA XIII.2.4**	**Effects on reproduction and growth rate with an invertebrate species**	
5.2 Results and discussion	Group 1: After 14 days of exposure, female survival of 92% was observed in the solvent control. Survival of 90%, 93% and 89% was observed in mysids exposed to the 0.015, 0.030 and 0.060 µg a.s./L concentrations, respectively, and no statistical differences were observed. 0.40, 0.31 and 0.44 offspring per female per reproductive day were counted in the 0.015, 0.030 and 0.060 µg a.s./L treatment, respectively. Statistically significant difference compared to the solvent control was observed in the 0.030 µg a.s./L treatment only (Bonferroni-test, α = 0.05) when compared to the control (0.59 offspring per female and day). However, since this reduction is not dose related, this effect is not considered biologically relevant. Group 2: After 28 days of exposure, female survival of 100% was observed in the solvent control. Survival of 85%, 100% and 95% was observed among mysids exposed to the 0.015, 0.030 and 0.060 µg a.s./L treatments, respectively. Reproductive success in group 2 was higher with 1.6, 1.8 and 2.0 offspring per female per reproductive day as counted in the 0.015, 0.030 and 0.060 µg a.s./L treatments, respectively. Statistically significant difference compared to the solvent control was observed at the 0.015 µg a.s./L treatment only (Bonferroni-test, α = 0.05). However, since this reduction is not dose related, this effect is not considered biologically relevant. The average total body length and the average dry body weight of male and female mysids were not significantly different when compared to the solvent control (Williams'-test, α = 0.05). Free ranging mysids: The total number of immature and mature mysids was not statistically different from the solvent control (Williams'-test, α = 0.05). The total free-ranging mysids in the 0.015, 0.030 and 0.060 µg a.s./L treatments were 511, 438 and 404, respectively, and was not statistically different from the solvent control (Williams'-test, α = 0.05). See also table A7_4_3_4-6 to table A7_4_3_4-8	
5.2.1 NOEC	60 ng a.s./L	
5.2. LOEC	> 60 ng a.s./L	
5.2.3 EC_{50} (EC_x)	-	
5.3 Conclusion	Based on nominal concentrations applied and the results of the statistical analyses of mysid survival, reproduction and growth, the LOEC was determined to be > 60 ng a.s./L (based on the most sensitive of the endpoints evaluated). The NOEC for Fipronil and mysids was determined to be 60 ng a.s./L. No validity criteria defined in the test guidelines.	
5.3.1 Reliability	1	

Section 7.4.3.4 Annex Point IIIA XIII.2.4	**Effects on reproduction and growth rate with an invertebrate species**	
5.3.2 Deficiencies	-	

Table A7_4_3_4-1: Preparation of TS Solution for Poorly Soluble or Volatile Test Substances

Criteria	Details
Dispersion	-
Vehicle	Acetone
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	The stock solution was prepared by placing 0.0302 g fipronil in a 100 mL volumetric flask and bringing it to volume with acetone.

Table A7_4_3_4-2: Dilution Water

Criteria	Details
Source	Natural filtered seawater
Salinity	20 ‰
Hardness	Not given
pH	Initial: 7.9
Ca / Mg ratio	Not given
Na / K ratio	Not given
Oxygen content	9.7 mg O_2/L
Conductance	27000 µmhos/cm
TOC	Not given
Holding water different from dilution water	No

Table A7_4_3_4-3: Test Organisms

Criteria	Details
Strain / Clone	*Americamysis bahia* (formerly: *Mysidopsis bahia*)

Source	Springborn Smithers Laboratories cultures (Lot No. 04A144) The brood stock was originally obtained from Aquatic BioSystems Inc., Fort Collins, Colorado, USA.
Age	Group 1: sexually mature, 21 days old Group 2: ≤ 24 hours old
Breeding method	Cultured in 76 L glass aquaria with a closed-loop recirculating filtration system providing seawater to the aquaria. Salinity: 19-21~~%~~ **‰** pH: 8.2-8.3 Dissolved Oxygen: 94-102% saturation Conductivity: 26000-32000 µmhos/cm Photoperiod: 16h light: 8 h dark Temperature: ~~26~~ **25**-26 °C
Kind of food (breed)	Live brine shrimp (*Artemia salina* nauplii), < 48 h old (post-hydration); Selco®
Amount of food (breed)	Not mentioned in the study report
Feeding frequency (breed)	Twice daily with shrimp; one feeding supplemented with Selco®
Pre-treatment	-
Feeding of animals during test	Like during breeding

Table A7_4_3_4-4: Test System

Criteria	Details
Test type	28 days life-cycle toxicity test under static conditions Juvenile mysids were released into each aquarium where they were exposed to water, sediment and test substance. Each exposure aquarium also contained five mysid pairing chambers used to house sexually mature male and female organisms. The pairing chambers were cylindrical glass jars (5.1 cm diameter, 10 cm high) containing two 2 cm holes covered with 350 µm Nitex® screens. At test initiation (day 0), and the time of sexual maturity (approx. day 14), adult mysids were transferred to the pairing chambers. The first group of sexually mature mysids (21 days old; group 1) were selected from the holding tank in the culture facility. At day 14, all mysids from group 1 were removed and a second group of sexually mature mysids was selected from the initial group of juvenile mysids exposed in the aquarium on day 0 and placed in the pairing chambers (group 2). Remaining juvenile mysids in each aquarium not used on day 14 in the mysid pairing were left for the remainder of the exposure to be free-ranging in the test aquarium. One male and one female were kept in each pairing chamber. Live young in each pairing chamber were counted, recorded and removed daily. A maximum of five pairing chambers were impartially suspended so as to not disturb the sediment layer in each test chamber.

Endpoints	Group 1 (parental mysids, 21 days old) exposed from day 0 to day 14: survival of females and reproduction Group 2 (≤ 24 hours old mysids) exposed from day 0 to day 28: survival, reproduction, growth (length and weight) and free-ranging population (number of adults, number of juveniles)
Sediment	Natural sediment, collected from Little Harbor Beach, Wareham, Massachusetts, USA; particles > 2.0 mm were removed Organic carbon: 2.7% Particle Size Distribution: 77% sand, 14% silt, 9% clay pH: 7.7 Moisture at 1/3 bar (water holding capacity): 19.2% Concentration of ammonia in the pore water: 18.6 mg N/L
Water	**Refer to Table A7.4.3.4-2**
Renewal of test solution	No
Volume of test vessels	19.5 L glass aquaria
Volume/animal	Approx. 0.75 L of sediment **(~1 cm layer)** and 16 L overlying water/50 mysids (≤ 24 h old) in the aquarium and 5×2 sexually mature mysids in the pairing chambers
Number of animals/vessel	50 mysids (≤ 24 h old) in ~~the~~ **each** aquarium and 5×2 sexually mature mysids in the **corresponding** pairing chambers
Number of vessels/concentration	4 replicates per treatment level **and solvent control** plus additional 6 replicates for the highest treatment level **(for analytical measurements)**
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test Conditions

Criteria	Details
Test temperature	25 ± 2 °C
Salinity	20 ± 3 ‰; adjusted during the exposure with deionized water to compensate for loss due to evaporation
Dissolved oxygen	5.5 – 7.6 mg O_2/L
pH	Initial: 7.9; during the test: 7.4 -8.0
Adjustment of pH	-
Aeration of dilution water	Aaeration with oil-free air
Quality/Intensity of irradiation	720-1250 lux
Photoperiod	16 h light: 8 h dark
Control of ammonia levels	< 0.10 – 1.0 mg N/L

Table A7_4_3_4-6: Summary of biological data for the water-sediment life-cycle exposure of mysids (*Americamysis bahia*) to fipronil – Group 1

Nominal concentration (ng a.s./L)	**Group 1,** Means (Standard Deviation)	
	Female % Survival [b]	# of offspring per Reprod. Day
Solvent control [a]	92 (14)	0.59 (0.14)
15	90 (12)	0.40 (0.15)
30	93 (12)	0.31 (0.11) [c]
60	89 (13)	0.44 (0.01)

[a] Replicate B of the solvent control was excluded from analysis due to contamination
[b] Female survival was adjusted for organisms lost due to impingement.
[c] Statistically different from the solvent control (Bonferroni T-test) but not considered biologically relevant.

Table A7_4_3_4-7: Summary of biological data for the water-sediment life-cycle exposure of mysids (*Americamysis bahia*) to fipronil – Group 2

Nominal concentration (ng a.s./L)	**Group 2,** Means (SD)					
	Female % Survival [b]	# of Offspring per Reprod. Day	Male Length (mm)	Male Weight (mg)	Female Length (mm)	Female Weight (mg)
Solvent control [a]	100 (0)	2.2 (0.31)	7.7 (0.36)	0.94 (0.14)	7.9 (0.26)	1.2 (0.14)
15	85 (10)	1.6 (0.22)	7.6 (0.32)	0.82 (0.21)	7.9 (0.28)	1.3 (0.08)
30	100 (0)	1.8 (0.36)	7.7 (0.42)	0.93 (0.15)	7.9 (0.40)	1.2 (0.30)
60	95 (10)	2.0 (0.33)	7.6 (0.26)	0.88 (0.07)	8.1 (0.20)	1.3 (0.18)

[a] Replicate B of the solvent control was excluded from analysis due to contamination
[b] Female survival was adjusted for organisms lost due to impingement.
[c] Statistically different from the solvent control (Bonferroni T-test) but not considered biologically relevant.

Table A7_4_3_4-8: Summary of biological data for the water-sediment life-cycle exposure of mysids (*Americamysis bahia*) to fipronil – free-ranging Mysids

Nominal concentration (ng a.s./L)	**Free-ranging Mysids,** Means (Standard Deviation)		
	# of Immature Mysids	# of Mature Mysids	Population (free-ranging)
Solvent control [a]	489 (82)	46 (10)	535 (83)
15	480 (104)	31 (3)	511 (106)
30	397 (143)	41 (10)	438 (151)
60	362 (71)	42 (10)	404 (61)

[a] Replicate B of the solvent control was excluded from analysis due to contamination

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 November 2007
Materials and methods	Agree with the applicant's version. **Revisions/amendments:** **3.4.9 Monitoring of TS concentration** : Water from each concentrations and solvent control (two replicates) was analyzed. Sediment samples were analyzed from the highest treatment level (auxiliary replicates) at the initiation of the test (day 0), on day 4, and weekly thereafter. Errors in Table A7.4.3.4-3 are corrected in bold and underlined. Additional information were included in Table A7.4.3.4-4 in bold and underlined.
Conclusion	Agree with the applicant's version. **Revisions/amendments:** **5.1 Materials and methods :** At test initiation *200 mysids (≤ 24 h old) per test concentration were released in aquariums (4 replicates per treatment group, 50 individuals per aquarium). In addition, 40 sexually mature mysids (20 pairs of 21-day old mysids) were transferred in 20 pairing chambers also placed in the exposure aquariums (five per aquarium) of each treatment level and the solvent control.* ~~At test initiation, 21-day old mature male/female pairs were transferred into each of five individual glass pairing chambers.~~ *These* 21-day old mature *organisms were used to evaluate mysid reproduction during the initial 14-day exposure (designated group 1).*
Reliability	1
Acceptability	The study is not acceptable. The organism exposure in this study was carried out in static conditions and results are based on nominal concentrations of the molecule at test initiation, which is representative of an intermittent release. As the release of this type of product is a continuous phenomenon through sewage treatment plant facilities, the NOEC obtained can't be used for biocidal risk assessment purposes.
Remarks	The method validation studies were conducted on freshwater. A method verification with filtered seawater is indicated but no data were presented. This deficiency does not call into question the validity of the study.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 7.4.3.5 **Annex Point IIIA, XIII.3.4**	**Effects on any other specific, non-target organisms (flora and fauna) believed to be at risk**

Section 7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment dwelling organisms**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A.7.4.3.5.1/01** XXXX Fipronil – Toxicity to midge (*Chironomus tentans*) during a 10-day sediment exposure. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA 850.1735	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2	
3.1.1	Lot/Batch number	Batch No. SEL/1121	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	Radiopurity: 98.3%	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	Water solubility: 3.78 mg/l	
3.1.6	Method of analysis	Total radioactivity measured byLiquid Scintillation Counting.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	64.2 µg/ml See table A7.4.3.5.1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	

Section 7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment dwelling organisms**

3.4	**Testing procedure**		
3.4.1	Dilution water	See Table A7.4.3.5.1-1	X
3.4.2	Test organisms	See Table A7.4.3.5.1-3	X
3.4.3	Test system	See Table A7.4.3.5.1-4	X
3.4.4	Test conditions	See Table A7.4.3.5.1-5	X
3.4.5	Duration of the test	10 days	
3.4.6	Test parameter	Mortality and behaviour daily. Growth (dry weight of midge larvae) at the end of the test, day 10.	
3.4.7	Sampling	At test initiation and at 24-hours interval thereafter	
3.4.8	Monitoring of TS concentration	No	**X**
3.4.9	Statistics	Descriptive statistics, Chi-Square test for survival data, Shapiro-Wilks-test for growth values	
		4. RESULTS	
4.1	**Limit test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable	
4.1.3	Nature of adverse effects	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance Nominal	µg/l: 6.3 | 13 | 25 | 50 | 100 | 200	
4.2.2	Actual concentrations of test substance	See Table A7.4.3.5.1-6	
4.2.3	Effect data (Mortality)	See Tables A7.4.3.5.1-6	
4.2.4	Concentration / response curve	None available	
4.2.5	Other effects	See Tables A7.4.3.5.1-6	
4.3	**Results of controls**		
4.3.1	Number/percentage of animals showing adverse effects	See Table A7.4.3.5.1.1-6	
4.3.2	Nature of adverse effects	See Table A7.4.3.5.1.1-6	

Section 7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment dwelling organisms**

4.4	**Test with reference substance**	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: Fipronil (BAS 350 I); radiolabelled material (^{14}C-fipronil, batch no. SEL/1121 2nd crop, purity: 98.3%). Test species: *Chironomus tentans*, egg masses collected from in-house culture; larvae 10 days old at test initiation. Test design: Static renewal system, test duration 10 days, 6 concentrations plus control and solvent control (acetone), each with 12 replicates (8 replicates for biological examination, 4 for the chemical analysis), 10 *Chironomus tentans* larvae added to each vessel (replicate). Water renewal at a rate of two test volumes per day. Endpoints: Mortality and behaviour daily. Growth (dry weight of midge larvae) at the end of the test, day 10. Test conc.: Control, 6.3, 13, 25, 50, 100 and 200 µg a.s./kg sediment (nominal). Test item mixed into the sediment (spiked-sediment test). Test conditions: 300 mL glass vessels filled with a 2 cm deep layer of sediment (natural sediment from a pond in Wareham, Massachusetts) and topped with 175 mL overlying water (laboratory well water), pH 6.3 – 6.9, oxygen content 4.0 – 8.9 mg/L, conductivity 180 – 190 µS/cm, feeding with flaked fish food suspension, water temperature 21 – 25 °C, light intensity 538 – 861 lux, photoperiod: 16 light :8 h dark. Analytics: The concentrations of the test item in sediment, overlying water and pore water were determined by measuring the total radioactivity using a Liquid Scintillation Counter (LSC). During the in-life phase of the study, samples of sediment, overlying water and pore water were collected and analyzed on test days 0, 4 and 10. Statistics: Descriptive statistics, Chi-Square test for survival data, Shapiro-Wilks-test for growth values.	
5.2	**Results and discussion**	Analytical measurements: Due to inconsistent results of both pore water and overlying water on test day 0, additional samples were taken from the sediment, pore water and overlying water on test day 4. The mean-measured concentrations of total ^{14}C-fipronil in the sediment were 7.6, 16, 33, 68, 140 and 290 µg/kg for the nominal treatment levels 6.3, 13, 25, 50, 100 and 200 µg/kg, respectively. These measured sediment concentrations of fipronil residues ranged from 120% to 150% of the nominal concentrations.	

Section 7.4.3.5.1 **Annex Point IIIA, XIII.3.4**	**Effects on sediment dwelling organisms**

		Measured concentrations of total ^{14}C-fipronil residue in pore water samples were similar between sampling intervals and followed the expected concentration gradient. The mean measured pore water concentrations calculated during this test were 0.10, 0.24, 0.47, 1.0, 2.1 and 4.2 µg/L. On test day 0, measured concentrations of total ^{14}C-fipronil residues in overlying water in the 50, 100 and 200 µg/kg treatment levels were 0.060, 0.26 and 0.10 µg/L, respectively. Measured concentrations of total ^{14}C-fipronil residue in the remaining treatment levels (6.3 to 25 µg/kg) were below the limit of detection (i.e. <0.056 µg/L). On day 10, measured concentration of total ^{14}C-fipronil residue in the 200 µg/kg treatment level was 0.13 µg/L and in the remaining treatment levels (6.3 to 100 µg/kg) below the limit of detection. It was established that ^{14}C-fipronil remained in association with the sediment and did not significantly partition to the overlying water. In addition, the concentrations of ^{14}C-fipronil in the pore water were in equilibrium with the sediment throughout the 10-day exposure. The biological results are based on mean-measured sediment and pore water concentrations. Biological results: Survival rates in the control and solvent control averaged 96% and 95%, respectively. The mean dry weight of the larvae in the two control groups was 1.41 mg. At test termination at day 10, mortality observed among midges in the 33, 68, 140 and 290 µg/kg treatment levels was 61, 100, 100 and 100%, respectively. The differences were statistically significant between these three groups and the pooled controls. The NOEC for midge survival was 16 µg/kg sediment and 0.24 µg/L pore water, respectively. The 10-day LC_{50} values for midge survival are 30 µg/kg sediment and 0.43 µg/L pore water, respectively. The NOEC for midge growth was 33 µg/kg sediment and 0.47 µg/L pore water, respectively. The 10-day EC_{50} values for midge growth were 50 µg/kg sediment and 0.73 µg/L pore water, respectively.
5.2.1	LC_0	Not recorded
5.2.2	10-day LC_{50} survival	30 µg/kg sediment
5.2.3	10-day EC_{50} growth	50 µg/kg sediment
5.2.3	10-day NOEC	16 µg/kg sediment
5.3	**Conclusion**	
5.3.1	Other conclusions	The NOECs for midge growth and survival were 33 µg/kg and 16 µg/kg sediment, respectively
5.3.2	Reliability	1

Section 7.4.3.5.1 Effects on sediment dwelling organisms **Annex Point IIIA, XIII.3.4**

5.3.3 Deficiencies	None	

Table A7.4.3.5.1-1 Dilution water

Criteria	Details
Hardness	52 mg/l as $CaCO_3$
Alkalinity	36 mg/l as $CaCO_3$
pH	6.0 – 8.5
Dissolved Oxygen	7.5 to 8.9 mg/l
Conductance	180 – 190 µmhos/cm
Holding water different from dilution water	No

Table A7.4.3.5.1-2 Natural Sediment

Criteria	Details
Source	Glen Charlie Pond, Massachusetts
Composition	2.8% organic carbon 94% sand 6% silt 0% clay
pH	5.7

Table A7.4.3.5.1-3 Test organisms

Criteria	Details
Species / Clone	*Chironomus tentans*
Source	XXXX
Age	10 days
Breeding method	Not recorded
Kind of food	Ground suspension Ziegler Brothers flaked fish food
Amount of food	Not recorded
Feeding frequency	Daily
Pretreatment	None
Feeding of animals during test	Yes

Table A7.4.3.5.1-4 Test system

Criteria	Details
Test type	Static
Renewal of test solution	2 overlying water per day (350 ml)
Volume of test vessels	275ml (100ml sediment + 175 overlying water)
Volume/animal	
Number of animals/vessel	10
Number of vessels/ concentration	12
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.4.3.5.1-5 Test conditions

Criteria	Details
Test temperature	23 ±1°C
Dissolved oxygen	> 8 mg/l
pH	7.5 to 7.6
Adjustment of pH	No
Aeration of dilution water	No
Light Intensity	538 – 861 Lux
Photoperiod	16 hour light. 8 hour dark

X

Table A7.4.3.5.1-6 Mortality data

nominal sediment concentration [µg a.s./kg]	**control**	**solvent control**	**6.3**	**13**	**25**	**50**	**100**	**200**
Mean-measured sediment concentration [µg a.s./kg]	**0**	**0**	**7.6**	**16**	**33**	**68**	**140**	**290**
Mean-measured pore water concentration [µg a.s./L]	**0**	**0**	**0.1**	**0.24**	**0.47**	**1.0**	**2.1**	**4.2**
Mortality [%]	4	5	5	6	61*	100*	100*	100*
Mean dry weight/larvae [mg]	1.41	1.41	1.42	1.33	1.48	--	--	--
Endpoints (measured sediment concentration [µg a.s./kg])								
LC_{50} (mortality)	30 (95% confidence limits: 28 – 32)							
NOEC (mortality)	16							
EC_{50} (growth)	50 (95% confidence limits: 48 – 51)							
NOEC (growth)	33							
Endpoints (measured pore water concentration [µg a.s./L])								
LC_{50} (mortality)	0.43 (95% confidence limits: 0.41 – 0.45)							
NOEC (mortality)	0.24							
EC_{50} (growth)	0.73 (95% confidence limits: 0.70 – 0.74)							
NOEC (growth)	0.47							

* = significantly different from the pooled controls (Chi-Square test for survival, Shapiro-Wilks-test, $\alpha = 0.05$)

Table A7.4.3.5.1-7 Validity Criteria for sediment freshwater invertebrate testing following guideline EPA OPPTS 850.1735

	Fulfilled	**Not fulfilled**
Test must start with third-instar larvae or younger animals	✓	
Average survival in the control at the end of test should be 70% or greater	✓	
Weight of control organisms at the end of the test should average at least 0.60 mg/larvae	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 13, 2006
Materials and methods	There are some inaccuracies with the study report: 3.4.1 In the Table A7.4.3.5.1-1 *Dilution water*: In compliance with the study report, pH range is of *7.5 to 7.6* 3.4.2 In the Table A7.4.3.5.1-3*Test organisms*: Information on amount of food is given in the study report : "the test midges will be 1.5 mL of fish food suspension at 4 mg/mL. 3.4.3 Table A7.4.3.5.1-4 *Test system* Number of vessels/ concentration: *12 (8 replicates for biological examination, 4 for the chemical analysis).* 3.4.4 Table A7.4.3.5.1-5*Test conditions*: pH range: *6.3 – 6.9* Dissolved oxygen range: *4 – 8.9 mg/L* Temperature: *21 – 25°C* 3.4.8 Concentrations in sediment have been measured by LSC at Day 0, 4 and 10. Results are based on the average of the 3 values. 5.1 ~~*Mortality and behaviour daily*~~. Mortality and abnormal behaviour were examined daily.
Results and discussion	Agree with applicant's version. It should be noted that temperature (21 – 25°C) and oxygen (maintained above 40% saturation) were lower than the recommended ranges (23 – 25 °C and dissolved oxygen concentration does not fall below 60 per cent of ASV).
Conclusion	The applicant does not make any conclusions. It should be noted that the exposure stage is L3 larvae, which is not recommended by OECD Guideline 218 and is likely not the most sensitive stage. In addition, as the test duration is of only 10 days, the effects on the whole larvae development and emergence of adults are not addressed in this study. Thus toxicity endpoints are probably underestimated. However, the used methodology (test system and analytical measurements) is well appropriate to investigate the effects of unstable chemical and according to the OECD Guideline 218 this study of 10 days is reliable to determine short-term data.
Reliability	Due to the deficiencies reported, the reliability indicator should be set to 2.
Acceptability	Acceptable as supportive short-term data.
Remarks	
	COMMENTS FROM …
Date	

Results and discussion
Conclusion
Reliability
Acceptability
Remarks

		Official use only
	2. REFERENCE	
1.1 Reference	**A.7.4.3.5.1/02** XXXX Chronic toxicity of BAS 350 I (Fipronil) to the non-biting midge *Chironomus riparius* - a spiked sediment study. (unpublished) (XXXX)	
1.2 Data protection	Yes	
1.2.1 Data owner	BASF SE	
1.2.2 Companies with letter of access	None	
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	OECD Guideline 218 (adopted 2004) "Sediment-water chironomid toxicity test using spiked sediment".	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	Fipronil (BAS 350 I)	
3.1.1 Lot/Batch number	Batch no. COD-000172	
3.1.2 Specification	See 3.1.3	
3.1.3 Purity	95.4%	
3.1.4 Composition of product	Not relevant	
3.1.5 Further relevant properties	Not relevant	

3.1.6 Method of analysis	HPLC/MS
3.2 Preparation of TS solution for poorly soluble or volatile test substances	A stock solution was prepared by dissolving 34.0 mg of BAS 350 I in 32.4 mL acetone (taking the purity into account) and a following dilution by adding 0.1 mL of the stock solution to 10 mL acetone (concentration of the second stock solution 0.1 mg / mL). For the test concentration of 0.2 µg/kg dry sediment, 11.43 µL of the second stock solution were taken and filled up to 10.0 mL acetone; this dilution was added to 80 g of quartz sand and mixed homogeneously. After an evaporation time of about 1 h under the fume hood, the spiked quartz sand was added to 620 g sediment and mixed again thoroughly. In order to include potentially remaining residues from the vessel in which the quartz sand and the test item were mixed, the vessel was rinsed with 100 mL deionised water, which were carefully added to the sediment. Afterwards, the moisture content of the sediment was approximately 30%. Then, 100 ± 2 g of the wet spiked sediment was added to each of five 600 mL glass beakers (one for chemical analysis and 4 for exposure). For the higher concentrations the exact same procedure was repeated using respective dilutions of the above-mentioned stock solution. The stock solution was clear without any visible precipitation. It was assured that the same concentration of solvent was used in the sediment of the solvent control. For the water control, the quartz sand without solvent was mixed into the sediment and 100 mL of water, as described above was added.
3.3 Reference substance	Lindane
3.3.1 Method of analysis for reference substance	No data
3.4 Testing procedure	
3.4.1 Dilution water	Reconstituted water (M4 according to Elendt) (table A7_4_3_5_1_2-1a) and artificial sediment (OECD 218; 2004) (table A7_4_3_5_1_2-1b) were used as media.
3.4.2 Test organisms	Non-biting midge (*Chironomus riparius*), collected from in house laboratory culture; first instar larvae (table A7_4_3_5_1_2-2)
3.4.3 Test system	The test system was allowed to stabilise for 2 days before addition of the larvae. The following definitions therefore apply: DAT: day after treatment. DAI: day after insertion of the larvae. For further details on the test system cf. table A7_4_3_5_1_2-3.
3.4.4 Test conditions	See table A7_4_3_5_1_2-4
3.4.5 Duration of the test	28 days (exposure)

3.4.6 Test parameter	**Development rate:** development time represents the time span between the insertion of the larvae (DAI 0) and the emergence of the adult midges. The development rate is the reciprocal of the development time (day^{-1}) and represents the portion of larval development taking place per day. **Emergence rate** is the ratio of the emerged insects (imagines) to introduced larvae. **NOEC** is the highest concentration of the test item at which no significant effects (on emergence or development rate or other observations) occur relative to the (water, solvent or pooled) control. The LOEC is the next higher concentration, i.e. the lowest concentration at which a significant effect was determined. **EC_{50}** is the concentration causing a 50% effect on the parameter under consideration. It is usually determined mathematically (e.g. via probit, logit or log-log analysis).	
3.4.7 Sampling	Before emergence of the first midge occurs observations on behaviour and mortality were made at least 3 times a week, and observed irregularities were recorded. From the onset of emergence observations on behaviour and mortality were made once each day. Furthermore, the emerged adults were removed from the vessels daily, and their number and sex was recorded.	
3.4.8 Monitoring of TS concentration	One additional vessel for each treatment group was set up for chemical analysis of sediment, overlaying water and pore water on DAT 2 (test initiation). The analysis of sediment, overlaying water and pore water on DAT 30 (test termination) for each treatment group, was conducted in the same test vessels as used for the biological assessments.	
3.4.9 Statistics	Statistical determination of the NOEC for emergence rate and development rate was done by analysis of variance (ANOVA) followed by Dunnett's Multiple t-test Procedure to test for significant differences between the treatment and the solvent control and the water control, too ($\alpha = 0.05$). The calculations were conducted on a PC using the commercial software package ToxRatPro, Version 2.10 (ToxRat® Solutions GmbH).	
	4. RESULTS	
4.1 Limit test	-	
4.1.1 Concentration	-	
4.1.2 Number/ percentage of animals showing adverse effects	-	
4.1.3 Nature of adverse effects	-	
4.2 Results test substance		

4.2.1 Initial concentrations of test substance Nominal	Based on the results of a non-GLP range finding test, target concentrations for the definitive GLP test were selected. The following target concentrations were selected: 0.2, 0.4, 0.8, 1.6 and 3.2 µg /kg dry sediment.	
4.2.2 Actual concentrations of test substance	The results of the chemical analyses of the test item in sediment, overlaying water and pore water are summarised in table A7_4_3_5_1_2-6. Analytical confirmation of the sediment concentration at DAT 2 by HPLC/MS yielded the following initially measured concentrations for BAS 350 I: < LoQ, < LoQ, 0.242, 0.412, 0.675, 1.61 and 2.60 µg/kg dry sediment. Hence, the recovery of the test item in the treated sediment ranged from 81.2 to 121.0%. DAT 30 measurements show the following concentrations for BAS 350 I: < LoQ, < LoQ, 0.201, 0.322, 0.623, 1.17 and 2.16 µg/kg dry sediment. The recovery of the test item in the treated sediment on DAT 30 ranged from 67.6 to 100.5%. Overlaying water concentrations ranged from < LoD to 36.5 ng a.i./L on DAT 2 and from 20.7 to 61.6 ng a.i./L on DAT 30. The pore water concentrations measured in the spiked sediments ranged from 62.0 to 235 ng a.i./L on DAT 2 and from < LoQ to 41.6 ng a.i./L on DAT 30.	X
4.2.3 Effect data (Mortality)	On DAI 13 (= day after insertion of the larvae), the first emerged midges were observed. Males always emerge earlier than females, which is a natural phenomenon in *C. riparius*. There was no indication for different effects on males and females. Therefore, and to improve statistical power, male and female data were pooled for the calculations. As recommended in OECD-guideline 218 the biological results are based on initial measured (DAT 2 = DAI 0) concentrations of BAS 350 I. The **NOEC** as determined by comparison with either the solvent control or the water control for development rate was **≥ 2.60 µg a.i./kg dry sediment** and for emergence rate **1.61 µg a.i./kg dry sediment** (ANOVA, Dunnett's Multiple t-test Procedure, $p < 0.05$). For emergence rate and development rate no EC_{50} could be calculated due to a lack of effects, but will be ≥ 2.6 µg a.i./kg dry sediment. See table A7_4_3_5_1_2-5 for individual values.	X
4.2.4 Concentration / response curve	A clear concentration-response relation was observed. The effects of test substance on the emergence rate per test substance concentration are plotted on p. 19 of the report.	
4.2.5 Other effects	-	
4.3 Results of controls		
4.3.1 Number/percentage of animals showing adverse effects	Emergence rate in the water controls was 0.90 and in the solvent control 0.867.	
4.3.2 Nature of adverse effects	-	

4.4 Test with reference substance	A study with the reference item lindane was completed in August 2009 (XXXX). The NOEC for the development rate was 2.0 µg/L (nominal) and for the emergence rate 2.0 µg/L (nominal). The results were within the expected range.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	*Test item:* BAS 350 I, batch COD-000172, purity 95.4% *Test species*: Non-biting midge (*Chironomus riparius*), collected from in house laboratory culture; first instar larvae. *Test guidelines*: OECD Guideline 218 (adopted 2004) Sediment-water chironomid toxicity test using spiked sediment. *Test parameters*: 600 mL glass vessels, containing about 100 g wet artificial sediment, 400 mL M4 water (Elendt medium), pH 7.80 – 8.53, oxygen content 8.00 – 9.05 mg/L (> 60% air saturation value), total hardness 2.85 mmol/L, conductivity 677 µS/cm (hardness and conductivity from bulk dilution water), ammonia at DAT 2: 0 mg/L and at DAT 30: 4.5 mg/L. Feeding with TetraMin, gentle aeration, water temperature 19.2 - 20.4°C during the test, light intensity 520 - 851 lux, day: night-rhythm 16:8 h. *Test design*: Static test; five concentrations: 0.2, 0.4, 0.8, 1.6 and 3.2 µg a.i./kg dry sediment (nominal target values), with four replicates, plus a solvent (acetone) control with six and a solvent free (water) control with four replicates. 20 *C. riparius* first instar larvae were added to each vessel; assessment of emergence rate and development rate. *Statistics*: Descriptive statistics, Dunnett's Multiple t-test Procedure ($\alpha = 0.05$)	**X**
5.2 Results and discussion	Validity criteria according to OECD guideline 218 are fulfilled (table A7_4_3_5_1_2-7). Analytical measurements of sediment concentrations of BAS 350 I by HPLC/MS on DAT 2 yielded the following values: 0.242, 0.412, 0.675, 1.61 and 2.60 µg a.i./kg dry sediment. At DAT 30 the analytical measurements of the sediment yielded the following values: 0.201, 0.322, 0.623, 1.17 and 2.16 µg a.i./kg dry sediment. Following the guideline the initial (DAT 2) measured sediment values are used for the statistical evaluation of the biological data. Overlaying water concentrations ranged from < LoD to 36.5 ng a.i./L on DAT 2 and from 20.7 to 61.6 ng a.i./L on DAT 30. The pore water concentrations measured in the spiked sediments ranged from 62.0 to 235 ng a.i./L on DAT 2 and from < LoQ to 41.6 ng a.i./L on DAT 30.	**X**
5.2.1 NOEC	The **NOEC** for **emergence rate** was **1.61 µg a.i./kg dry sediment,** and for development rate ≥ 2.60 µg a.i./kg dry sediment. For emergence rate and development rate an EC_{50} could not be calculated due to a lack of effects. Therefore, the EC_{50} for both parameters is ≥ 2.6 µg a.i./kg dry sediment	**X**
5.3 Conclusion	The **NOEC** for **emergence rate** was **1.61 µg a.i./kg dry sediment,** and for development rate ≥ 2.60 µg a.i./kg dry sediment.	**X**

5.3.1	Other conclusions	-
5.3.2	Reliability	1
5.3.3	Deficiencies	None

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29/03/2010
Materials and methods	Agree with applicant's version
Results and discussion	The applicant's version is adopted with the following revisions/amendments : 4.2.2 Actual concentrations of test substance *Analytical confirmation of the sediment concentration at DAT 2 by HPLC/MS yielded the following initially measured concentrations for BAS 350 I: < ~~LoQ~~* LOD*, < ~~LoQ~~* LOD*, 0.242, 0.412, 0.675, 1.61 and 2.60 µg/kg dry sediment. Hence, the recovery of the test item in the treated sediment ranged from 81.2 to 121.0%. DAT 30 measurements show the following concentrations for BAS 350 I: < ~~LoQ~~* LOD*, < ~~LoQ~~* LOD*, 0.201, 0.322, 0.623, 1.17 and 2.16 µg/kg dry sediment. The recovery of the test item in the treated sediment on DAT 30 ranged from 67.6 to 100.5%.* *Overlaying water concentrations ranged from < LoD to 36.5 ng a.i./L on DAT 2 and from ~~20.7~~* 19.0 *to 61.6 ng a.i./L on DAT 30.* *The pore water concentrations measured in the spiked sediments ranged from 62.0 to 235 ng a.i./L on DAT 2 and from < LoQ to 41.6 ng a.i./L on DAT 30.* The test item was found in quite high quantities in the controls on DAT 2, i.e. 394 and 93 ng a.i./L in the water control and solvent control, respectively. The explanation of this finding is cross contamination during handling in preparation for chemical analysis. 4.2.3 Effect data (Mortality) ~~*As recommended in OECD-guideline 218 the biological results are based on initial measured (DAT 2 = DAI 0) concentrations of BAS 350 I.*~~ Due to the fact that the recovery of the test item in the treated sediment on DAT 30 for the nominal concentrations of 1.6 and 3.2 µg/kg/kg dry sediment is calculated to be 73.1%, 67.5% respectively, the biological results are based on the mean measured concentration of BAS350 I between DAT 2 and DAT 30. *The **NOEC** as determined by comparison with either the solvent control or the water control for development rate was **≥ ~~2.60~~ 2.38 µg a.i./kg dry sediment** and for emergence rate **~~1.61~~ 1.39 µg a.i./kg dry sediment** (ANOVA, Dunnett's Multiple t-test Procedure, p < 0.05). For emergence rate and development rate no EC_{50} could be calculated due to a lack of effects, but will be ≥ ~~2.6~~ 2.38 µg a.i./kg dry sediment.*
Conclusion	The applicant's version is adopted with the following revisions/amendments : 5.1 Materials and methods *Test design: Static test; five concentrations: 0.2, 0.4, 0.8, 1.6 and 3.2 µg a.i./kg dry sediment (nominal target values), with four replicates, plus a solvent (acetone) control with six and a solvent free (water) control with four replicates.* The test system was allowed to stabilise for 2 days before addition of the larvae. *20 C. riparius first instar larvae were added to each vessel; assessment of*

	emergence rate and development rate. 5.2 Results and discussion …… ~~*Following the guideline*~~ *the initial (DAT 2)* The mean *measured sediment values* between DAT 2 and DAT 30 *are used for the statistical evaluation of the biological data…..* 5.2.1 NOEC *The **NOEC** for **emergence rate** was **~~1.61~~ 1.39 µg a.i./kg dry sediment,** and for development rate ≥ ~~2.60~~ 2.38 µg a.i./kg dry sediment. For emergence rate and development rate an EC_{50} could not be calculated due to a lack of effects. Therefore, the EC_{50} for both parameters is ≥ ~~2.6~~ 2.38 µg a.i./kg dry sediment* 5.3 Conclusion *The **NOEC** for **emergence rate** was **~~1.61~~ 1.39 µg a.i./kg dry sediment,** and for development rate ≥ ~~2.6~~ 2.38 µg a.i./kg dry sediment.*
Reliability	1
Acceptability	Acceptable
Remarks	

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7_4_3_5_1_2-1a: Test water

Trace nutrients	(mg/L)	Macro nutrients	(mg/L)
H_3BO_3	2.8595	$CaCl_2$	221.850
$MnCl_2$	0.2294	$MgSO_4$	60.223
LiCl	0.306	KCl	5.800
RbCl	0.071	$NaHCO_3$	64.800
$SrCl_2$	0.09044	Na_2SiO_3	4.300
NaBr	0.016	$NaNO_3$	0.274
Na_2MoO_4	0.05364	KH_2PO_4	0.143
$CuCl_2$	0.01344	K_2HPO_4	0.184

$ZnCl_2$	0.0130		
$CoCl_2$	0.00546		
KI	0.00325	**Vitamins**	**(µg/L)**
Na_2SeO_3	0.00219	Thiamine	75.00
NH_4VO_3	0.00058	B_{12}	1.00
Na_2EDTA	2.2582	Biotin	0.75
$FeSO_4$	0.5443		

Table A7_4_3_5_1_2-1b: Test sediment

Sediment characterisation	**Details**
Particle size distribution	The artificial substrate as described in OECD guideline 218 is used as sediment. It is composed as follows: ca. 5% finely ground sphagnum peat ca. 20% kaolin clay (kaolinite content > 30%) ca. 0.75% $CaCO_3$ (Merck 2069) ca. 75% quartz sand (ISS-0-VAC, Fa. Gebr. Willersinn) > 80% has a particle size > 0.09 and < 0.18 mm
Organic carbon (%)	5% organic matter (sphagnum peat)
Water content (%)	The moisture content was approximately 30%
pH	The pH was determined to be 6.69
Cation exchange capacity	No data
TOC (Total organic carbon)	No data

Table A7_4_3_5_1_2-2: Test organisms

Criteria	**Details**
Strain	Non-biting midge *Chironomus riparius*
Source	House laboratory culture, which was established with egg-ropes obtained from the Zoological Institute of the J.W. Goethe university in Frankfurt am Main, Germany.
Age (at start of the study)	First instar larvae not older than 2 days
Breeding method	No data
Kind of food	Commercially available fish food, TetraMin
Amount of food	The food was finely ground and suspended in M4 water. An amount of food suspension corresponding to about 0.25 - 1.0 mg TetraMin per larva per day (i.e. 5 - 20 mg/vessel/day) was added for each day up to DAI 27. Depending on the smell of the overlaying water and the amount of green algae grown in the test

	vessels, feeding was adjusted or completely stopped
Feeding frequency	Daily
Pretreatment	Two days prior to addition of the larvae, fresh egg masses were collected from the culture and transferred to small vessels with standard M4-water, containing a small amount of green algae. About two days later the first larvae start hatching. Two days after preparing the vessels with spiked sediment, 20 1st instar larvae (not older than 2 days) were added to the test vessels. During addition of the larvae and for about 24 h afterwards, the aeration was stopped to give the larvae the opportunity to settle into the sediment.
Feeding of animals during test	Yes

Table A7_4_3_5_1_2-3: Test system

Criteria	Details
Static test	Test glass vessels were covered to reduce evaporation and to avoid the escape of emerging midges. Test vessels contained 100 +/- 2 g wet artificial sediment and ca. 400 mL M4 water (Elendt medium) corresponding to a water layer of about **7.5 or** 8.0 cm.
Volume of test vessels	Glass vessels, 600 mL
Volume water/animal	400 mL / 20 larvae
Number of animals/vessel	20 larvae / vessel
Number of vessels/ concentration	For each test concentration and the solvent free (or water) control four replicates were used. For the solvent control 6 vessels were employed. One additional vessel for each treatment group was set up for chemical analysis on DAT 2.
Test concentrations (nominal)	0.2, 0.4, 0.8, 1.6 and 3.2 µg /kg dry sediment
Test performed in closed vessels due to significant volatility of TS	No

X

Table A7_4_3_5_1_2-4: Test conditions

Criteria	**Details**
Test temperature of the overlying water	19.2 - 20.4°C during the test
Dissolved oxygen of the overlying water	8.00 - 9.05 mg/L (> 60% air saturation value)
pH of the overlying water	7.80 – 8.53
Ammonium	ammonia at DAT 2: 0 mg/L and at DAT 30: 4.5 mg/L
Adjustment of pH	No
Aeration of dilution water	gentle aeration of the water was provided to each vessel via a glass Pasteur pipette of which the outlet was situated a few cm above the sediment.
Quality/Intensity of irradiation	520 - 851 lux

Photoperiod	16:8 light-dark-cycle

Table A7_4_3_5_1_2-5: Emergence rate (ER) and development rate (DR) with their respective standard deviations (SD)

Initial measured concentration [µg a.i./kg d.s.]	ER (SD)	DR (SD)
control	0.9000 (0.0408)	~~0.0627~~ **0.0626** (0.0038)
solvent control	0.8667 (0.0683)	~~0.0622~~ **0.0621** (0.0022)
0.242	0.8375 (0.1109)	~~0.0619~~ **0.0617** (0.0032)
0.412	0.8125 (0.1031)	~~0.0645~~ **0.0642** (0.0018)
0.675	0.7500 (0.0408)	~~0.0631~~ **0.0630** (0.0003)
1.61	0.8000 (0.0707)	~~0.0634~~ **0.0630** (0.0041)
2.60	0.3750 (0.0289)*	~~0.0627~~ **0.0627** (0.0019)

X

d.s. = dry sediment, * indicates significant difference from the solvent and water control

Table A7_4_3_5_1_2-6: Measured concentrations (in µg a.i./kg dry sediment) of BAS 350 I in the sediment, overlaying water, and pore water at different times after application

Nominal target concentration [µg/kg d.s.]	DAT 2	DAT 30
	measured conc. [µg/kg d.s.]	measured conc. [µg/kg d.s.]
0 (water control)	< LoD	< LoD
0 (solvent control)	< LoD	< LoD
0.2	0.242	0.201
0.4	0.412	0.322
0.8	0.675	0.623
1.6	1.61	1.17
3.2	2.60	2.16

d.s. = dry sediment LoD = Limit of detection

Nominal sediment conc. [µg a.i./kg dry weight]	Time [DAT]	Overlaying water [ng a.i./L]	Pore water [ng a.i./L]
0 (water control)	2	< LoD	*394**
	30	< LoD	< LoD

0 (solvent control)	2	< LoD	*93.0**
	30	< LoD	< LoD
0.2	2	< LoD	190
	30	20.7	< LoD
0.4	2	5.83	134
	30	19.0	7.4
0.8	2	11.8	62.0
	30	20.3	13.7
1.6	2	10.3	69.7
	30	27.6	25.2
3.2	2	36.5	235
	30	61.6	41.6

* these analytical findings in the controls above LoD (5 ng/L) are probably caused by cross-contamination during sample handling, for further explanation see page 17 of the report.
LoD = Limit of detection

Table A7_4_3_5_1_2-7: Validity criteria according to OECD guideline 218

	Fulfilled	Not fulfilled
The emergence of the controls was ≥ 70%	**X**	
Emergence in the controls occurred between 12 and 23 days after insertion of the larvae	**X**	
The oxygen concentration was ≥ 60% of the ASV (air saturation value)	**X**	
The pH of the water was in the range 6 to 9	**X**	
The water temperature did not differ more than ± 1.0°C	**X**	

Section 7.4.3.5.2 **Annex Point IIIA, XIII.3.4**	**Aquatic plant toxicity**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.4.3.5.2/01** XXXX MB 46030 Toxicity to Duckweed, *Lemna gibba* (XXXX)	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA FIFRA Test Guidelines 122-2 and 123-2 (1982)	
2.2	**GLP**	Yes	
2.3	**Deviations**	1 The study protocol states that fronds are to be dried at 70°C for a minimum of two days. During this study, fronds were dried at 100°C for 3 days due to oven availability. Fronds were considered adequately dried for accurate dry weight determination. 2 The study protocol states that the analytical samples will be removed from the test solutions prior to dispersion to the test vessels. During this study, the analytical samples of the test solutions were removed after the solutions were transferred to the test vessels to maintain sterile conditions within the test solutions. 3 The study protocol states that a single analytical sample of each test concentration will be analyzed at test initiation and termination. During the study, because the definitive test was conducted with a single test concentration, triplicate samples of the test solution were analyzed at test initiation and termination. In the opinion of the performing laboratory these deviations did not affect he results of the study.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	fipronil	
3.1.1	Lot/Batch number	6ADM93	
3.1.2	Specification	As given in section 2	
3.1.3	Purity	96.1 % w/w	
3.1.4	Further relevant properties	None	

Section 7.4.3.5.2 **Annex Point IIIA, XIII.3.4**	**Aquatic plant toxicity**

3.1.5	Radiolabelling	No	
3.1.6	Method of analysis	HPLC with UV detection	
3.2	**Reference substance**	None	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing/estimation procedure**	Test item: M&B 46030 (fipronil) batch no 6ADM93, purity 96.1, described as a white powder	
		Test organism: the freshwater vascular plant used in this study was the duckweed *lemna gibba* strain G3, which was obtained from the university of California and maintained in stock culture at the performing laboratory.	
		The culture medium used was Hoagland's medium prepared with deionised water and adjusted to pH 5.0 ± 0.1 with 0.10N sodium hydroxide after autoclaving. Representative samples of the source of the deionised water were analysed for the presence of pesticides, PCB's and toxic metals. None of these compounds were detected at concentrations that are considered toxic in any of the water samples analysed. A representative sample of the medium was analysed monthly for total organic carbon concentration and was determined to range from 2.0 to 2.7 mg/l.	
		The stock cultures were maintained in an environmental chamber designed to maintain the following conditions for a minimum of seven days before testing: Temperature: 24 – 25°C pH range: 5.0 – 6.2 Illumination: continuous at approx 4000 – 4600 lux.	X
		Stock cultures were transferred to fresh medium approximately once weekly. The fronds used to initiate the toxicity test had been transferred seven days prior to testing.	
		Based on the results of a range-finding test a nominal test concentration of 0.20mg/l was selected for the definitive exposure. A 20 mg/l primary stock solution was prepared by dissolving 0.5203 of test material in 25ml acetone. This stock solution was then diluted to prepare the test solution.	X
		Sterile 270 ml crystallizing dishes, three per treatment level and the controls, were conditioned prior to use by rinsing with the appropriate solution. Two sets of control vessels were also established which contained the medium and were maintained under the same conditions as the treatment level vessels but contained no fipronil. One set of control vessels contained the maximum amount of acetone present in each test vessel and was designated the solvent control. All test vessels were covered with an inverted, sterile, glass Petri dish.	X

Section 7.4.3.5.2 Annex Point IIIA, XIII.3.4	**Aquatic plant toxicity**	
	Approximately 15 minutes after the test solution was prepared and added to the test vessels, an inoculum of 5 plants with 3 fronds each was aseptically introduced into each vessel. Test vessels were then randomly placed on a single shelf within an environmental chamber. At 3-day intervals fronds were counted and observations were made. At test termination, test day 14 frond densities and plant biomass for each replicate were determined. Fronds were counted and removed from each vessel, blotted dry and transferred to pre-treated aluminium pans. Fronds were dried at 100C for three days prior to dry weight determination.	
	4. RESULTS	
4.1 Experimental data		
4.1.1	**Preliminary testing** : A preliminary range-finding exposure was conducted at nominal concentrations of 0.0015, 0.015 and 0.15 m/l.. No significant effects were found in this study so the concentration for the definitive test was selected at 0.20mg/kg	
4.1.2	**Test solution concentrations** : Analysis of the test solutions at test initiation averaged 7.9% of nominal. At test termination measured concentrations from the treatment level averaged 21% of nominal concentration. Based on the decline of fipronil concentration in the test solutions over the 14 days test period, the results of this study are based on the measured concentrations obtained on day 0.	
4.1.3	**Frond production/observation**: During this study fronds exposed to the treatment level displayed treatment related effects (e.g. slightly chlorotic). Fronds exposed to the controls appeared normal throughout the exposure period. At test termination, the control and solvent control cultures averaged 425 and 402 fronds per replicate, respectively. The control and solvent control data were not statistically different and pooled for further analyses. Frond production in the treatment level averaged 382 fronds per replicate which was statistically reduced when compared to the pooled control data (414 fronds per replicate). Based on these results, the 14 day NOEC for frond density was determined to be < 0.16mg/l Based on frond density, the 14 day EC50 value was estimated to be > 0.16 mg/l. The treatment frond growth was reduced by 7.7% was compared to the pooled control data.	X
4.1.4	**Dry Frond weight**: Dry frond weights for the treatment level tested averaged 0.0901g. The control and solvent control cultures averaged 0.0936 and 0.0720g at test termination. The control and solvent control biomass data were not statistically different and therefore polled for further analyses.. Statistical analysis of the treatment data demonstrated that biomass in the treatment level was not significantly different when compared o the pooled control data. Based of this data the 14 day NOEC was determined to be 0.16mg/l	

Section 7.4.3.5.2 **Annex Point IIIA, XIII.3.4**	**Aquatic plant toxicity**

		5. APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	Test item M&B 46030 (fipronil) batch no 6ADM93, purity 96.1. Test organisms: fresh water duckweed *lemna gibba*, inoculum 5 plants with 3 fronds each. Test conditions Medium: Hoaglands. Temperature: 24 – 25°C pH range: 5.0 – 6.2 Illumination: continuous at approx 4000 – 4600 lux Exposure regime: static exposure over 14 days. Frond number was counted on days 3, 6, 9, 12 and 14 in each vessel. Following a range finding test the definitive test was conducted in a limit test designed with the nominal concentration of 2000 µg a.i/l. An untreated control and a solvent control (acetone at 100µl/l) were run in parallel. Each group consisted of 3 replicate vessels. Chemical analysis was performed at 0 and after 14 days
5.2	**Results and discussion**	The results of this study are summarised in terms of initial measured concentrations which averaged 79% of nominal. At test termination, measured concentrations averaged 21% of nominal. The mean measured concentration was approximately 50% of nominal. Any effects of the test item were determined in comparison to the pooled control. No significant effect of the test item was observed on the biomass produced by the plants through the 14 days exposure period. Small but statistically significant differences to the pooled control (<10%) were observed in the frond number at day 14. From day 9 the plants exposed to the test item were considered to be slightly chlorotic. The 14-d EC50 (biomass) and the 14-d EC50 (frond number) were empirically estimated to be above 160 µg a.i/l the initial measured concentration tested. The 14-d NOEC based on biomass was reported at 160 µg a.i/l.
5.3	**Conclusion**	The 14-d EC50 of fipronil in *Lemna gibba* is above 160 µg/l (limit test, initial measured concentration).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A7.4.3.5.2-1 Results

Initial measured concentration (µg/l)	Mean frond number per replicate (± SD)					Mean Dry weight mg (± SD)
	Day 3	Day 6	Day 9	Day 12	Day 14	
0 (control)	38±4	98±6	194±10	353±8	425±17	93.6±22.1
0 (solvent control)	42±3	94±10	181±6	329±8	402±24	72.0±7.8
0 (pooled control)	40±3	96±7	187±10	332±7	414±22	82.8±19.0
160	37±4	90±3	184±11[a]	327±13[a]	382±15[a]	90.1± 45.6

[a] plants were considered slightly chlorotic

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 16, 2006
Materials and methods	Applicant's version is adopted with the following revisions: 1.1 Date of the reference study (1993) 3.3 This section needs to be revised: *1)* There are some inaccuracies with the study report on the conditions of culture (3.3 second paragraph): *" The stock cultures were maintained in an environmental chamber designed to maintain the following conditions for a minimum of seven days before testing:* *Temperature*: 25 ± 2°C *pH range:* 5.0 ± 0.1 *Illumination: continuous at approx* 3800 - 5400 *lux."* *2)* *Based on the results of a range-finding test*, the definitive test was conducted as a Tier 1 test at a single nominal test concentration of 0.20mg/l*. A 20 mg/l primary stock solution was prepared by dissolving 0.5203* g *of test material in 25 ml acetone.* *3)* Each replicate test vessel contained 100 mL of the test medium*.One set of control vessels contained the maximum amount of acetone present in each test vessel* (100 µL/L) *and was designated the solvent control.* There are deviations from the OECD draft guideline 221: - As deviation from the nominal and measured initial concentrations is greater than ± 20%, analysis of the results should be based on the time-weighted mean and not on the measured concentrations obtained on day 0. Since the protocol does not allow to determinate the time-weighted mean, the results should be based on the geometric mean of the start and end of test concentrations i.e. 0.081 mg/L. - The pH of growth medium for *Lemna gibba* should be of 7.5 ± 0.1The light intensity was lower than the recommended range (6500 – 10000 lux). - As this test is comparable to a limit test at nominal concentration of 0.2 mg/L, the number of treatment replicates should be of 6.
Results and discussion	Applicant's version is adopted with the following revisions: 4.1.1 … *0.0015, 0.015 and 0.15 ~~m/l.~~* mg/L. 4.1.2 *Analysis of the test solutions at test initiation averaged ~~7.9%~~* 79% *of nominal."* The applicant does not discuss the results. No deficiencies reported. As this limit test shows a reduction of frond density of 7.7%, further testing would have to be performed. In addition, as the test substance cannot be maintained over the test duration, a semi-static test regime was recommended or at least an analytical determination of the test concentration over the test time at regular intervals. These methods

	would have allowed a better estimation of the exposure to fipronil and of toxic endpoints.
Conclusion	As it is not possible to determine a significant NOEC value, this test design does not fulfil the requirements of the directive. Nevertheless, an $EC_{10} > 0.081$ mg/L can be derived of results on frond density and regarded as the NOEC.
Reliability	2 in taking the geometric mean measured concentrations into account.
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5 **Annex Point IIA, VII**	**Effects on terrestrial organisms**

General comment on studies summarized in this section. The use of fipronil as Goliath Gel does not lead to any concern regarding potential exposure of terrestrial organisms to fipronil because the very particular indoor use of Goliath Gel. Since exposure in the terrestrial compartment is negligible, there is no requirement to include summaries of ecotoxicologocial studies with terrestrial species according to the Biocides Directive and associated guidance documents. However, since a comprehensive database of tests with fipronil on terrestrial non-target species is available, summaries of some key tests are included here voluntarily by the notifier to provide basic information for a better characterization of the toxicity profile of fipronil to terrestrial organisms.

Section A7.5.1 **Annex Point IIA, VII**	**Terrestrial toxicity, initial tests**

Section A7.5.1.1 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A.7.5.1.1/01** XXXX Effects of MB046030 on the activity of the soil microflora in the laboratory (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OIECD Guideline No 216 and 217	
2.2	**GLP**	Yes	

Section A7.5.1.1 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**

2.3	**Deviations**	The protocol requires that the concentration of ammonium, nitrate and nitrite be determined on day 0 within 6 hours of the initiation of the test. In this study the samples were taken within 6 hours but were stored overnight before analysis. This is presumed to have no effect on the study as the samples were refrigerated to prevent metabolic activity which could have affect the content of the nitrogen species. The protocol requires that the water content will be 40 – 60% of the maximum water holding capacity of the soil. The soil water content of one treatment group (fipronil at 0.133 mg/kg) in the carbon transformation test was measured at 39% of the maximum at day. This was considered to be an anomalous result as the water content throughout the remainder of the test was 42%. The concentration of fipronil in the soil nitrogen turnover test was 0.666 mg/kg rather than 0.667mg/kg. This variation is considered irrelevant	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Technical fipronil	
3.1.1	Lot/Batch number	01111701	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	98.6% w/w	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not applicable	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**		
3.3	**Reference substance**	Dinoterb (The inhibition of soil respiration and nitrogen turnover by Dinoterb are determined at this laboratory at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly).	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**		
3.4.1	Culture medium	The soil used in ths study was taken from a field to which no pesticides, organic or mineral fertilizers had been applied for 12,6 and 3 months respectively	
3.4.2	Inoculum / test organisms	Naturally occurring soil microfauna	

Section A7.5.1.1 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**	
3.4.3 Test system	Plastic boxes each containing approximately 1000g and 500g soil were used for the carbon transformation and nitrogen transformation tests respectively. The boxes were covered by perorated lids to enable a slight but sufficient air exchange. 25mg of fipronil was diluted to 50 ml in acetone which was applied to 15g quartz sand at rates calculated to give the correct application rate when mixed into the soil. The acetone was allowed to evaporate before the sand was incorporated.	
3.4.4 Test conditions	Temperature: 19.6 – 21.9 °C Light: Darkness Water: At the start of the test and weekly thereafter, the moisture during storage was controlled by adding deionised water and adjusted to approximately 40 to 45% of the maximum water-holding capacity at the time of application	
3.4.5 Duration of the test	28 days	
3.4.6 Test parameter	Carbon Transformation Test : Glucose induced respiration rate Nitrogen Mineralization and Nitrification Test nitrogen content	
3.4.7 Analytical parameter	Carbon Transformation Test : The soil samples (100g) were taken and mixed with 5 g/kg (moist soil) of glucose (2ml of a solution of 25o g/l). The amount of glucose had been determined to give the highest respiration rates (Determined on a batch of the test soil). The glucose amended soil samples were incubated at 20±2°C. The carbon dioxide released or the oxygen consumption were measured up to 24 consecutive hours using the BSB Sensomat system. For calculation of short term respiration, usually the linear part of the respiration curve after 2 hours up to 14 hours were used. Nitrogen Mineralization and Nitrification Test For the determination of nitrogen content, soil samples were taken within 6 hours after application and afterwards on each sampling date (7, 14 and 28 days). The nitrogen content was determined in each sample of treated and control soils. The soil samples were stored at 4±4°C overnight and extraction was done using 0.1M KCl solution. For the extraction approximately 30g soil was suspended in 100ml KCL solution and agitated for one hour. The suspension was centrifuged and the extracts filtered. The ammonium content was determined by means of a ion-sensitive electrode. Nitrate and nitrite were determined by ion chromatography.	

Section A7.5.1.1 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**	
3.4.8 Sampling	0 (within 6 hours), 7, 14 and 28 days	
3.4.9 Monitoring of TS concentration	None	
3.4.10 Controls	Untreated samples	
3.4.11 Statistics	Data were tested for normality and homogeneity of variance using R/S Test and Bartletts Test. Student t test was used for analysis	
	4. RESULTS	
4.1 Preliminary test	Not applicable	
4.1.1 Concentration		
4.1.2 Effect data		
4.2 Results test substance		
4.2.1 Initial concentrations of test substance	0.133 and 0.667 mg/kg	
4.2.2 Actual concentrations of test substance	0.133 and 0.667/0.666 mg/kg	
4.2.3 Growth curves	Not applicable	
4.2.4 Cell concentration data	Not applicable	
4.2.5 Concentration / response curve	Not applicable	
4.2.6 Effect data	See Tables A7.5.1.1-4 and A7.5.1.1-5 and Figures A7.5.1.1-1 and A7.5.1.1-2	
4.2.7 Other observed effects	None	
4.3 Results of controls		
4.4 Test with reference substance	Not part of this study. (The inhibition of soil respiration and nitrogen turnover by Dinoterb are determined at this laboratory at least once a year as a means of assuring that the laboratory test conditions are adequate and have not changed significantly).	
4.4.1 Concentrations		
4.4.2 Results		
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The study tested the effects of fipronil applied at 0.133 and 0.667 mg/kg soil dry weight on the carbon mineralization and the soil nitrogen turnover of the soil microflora in the laboratory. The test concentrations corresponded to a field application rate of 100 g fipronil per hectare and the 5-fold of this application (500 g/ fipronil per hectare. The carbon mineralization and the soil nitrogen turnover were determined in two different experiments.	

Section A7.5.1.1 **Annex Point IIA, VII.7.4**	**Inhibition to microbiological activity**

		Three treatment groups (control and the test item at two concentrations) with 3 or 4 replicates were each incubated at 20±2°C in the dark. At different sample times, the soil respiration and the soil nitrogen content was determined.	
5.2	**Results and discussion**	Soil Respiration Rate The differences between the respiration rates of fipronil treated and control soil samples were 2.11% for 0.133 mg fipronil/kg soil and 6.82% for the test concentration of 0.667 mg/kg at 28 days after application. According to OECD Guideline, no long term effects occur, if the deviation of the soil respiration rates from the control is less than 25% at 28 days after application. The variation between replicate control samples was 13.15% at day 28 after application being lower than the validity criterion of the OECD Guideline 217 of 15%. Soil Nitrogen Turnover The differences between the soil nitrate content of fipronil treated and control soil samples were none (0%) for 0.133 mg fipronil/kg soil and 4.40% for the test concentration of 0.667 mg fipronil/kg soil 28 days after application. According to OECD Guideline 216 no long-term effects occur, if the deviation of the soil nitrate content from the control is less than 25% at 28 days after application. The variation between replicate control samples was 8.41% at day 28 after application being clearly lower than the validity criterion of the OECD Guideline 216 of 15%. The study was valid since variation of the replicate control samples was less than 15% and the toxic standard Dinoterb treated soils (tested once a year) differed more than 25% from the control in the carbon mineralization test and the soil nitrogen turnover test. The potential impact of fipronil on the soil's microbial community is considered negligible at concentrations up to 0.667 mg of fipronil per kg soil dry weight.	
5.3	**Conclusion**		
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A7.5.1.1-1 Preparation of TS Solution for Poorly Stable or Volatile Test Substances

Criteria	Details
Dispersion	
Vehicle	Acetone
Concentration of vehicle	100%
Vehicle control performed	Yes
Other procedures	No

Table A7.5.1.1-2 Test system

Criteria	Details
Culturing apparatus	Plastic boxes containing 100g (carbon transformation test) or 500g (nitrogen turnover test) soil
Number of culture flasks/concentration	3 (carbon transformation test) or 4 (nitrogen turnover test)
Aeration device	Not applicable
Measuring equipment	pH: pH meter Water content:
Test performed in closed vessels due to significant volatility of TS	Not applicable

Table A7.5.1.1-3 Test conditions

Criteria	Details
Test temperature	19.6 – 21.9°C
pH	5.9 – 6.2
Water contents	39 – 44% of maximum water holding capacity

Table A7.5.1.1-4 Effect of Fipronil on soil respiration (Mean values)

Treatment Group	Time after initiation of the test (days)												
	0			7			14			28			
	Resp	SD	%	Resp	SD	%	Resp	SD	%	Resp	SD	%	Sig
control	1.113	0.03	-	1.235	0.12	-	1.196	0.03	--	1.232	0.09	-	-
0.133mg/kg fipronil	1.263	0.18	13.5	1.212	0.01	1.94	1.052	0.23	-12	1.206	0.06	2.11	Ns
0.667mg/kg fipronil	1.420	0.07	27.6	1.275	0.06	3.16	1.351	0.03	13	1.316	0.05	6.82	ns

Resp: respiration (mg CO_2/100g dry weight) mean of three samples
SD Standard deviation
%percentage variation from the corresponding control
Sig Significance (according to Student – t Test) from corresponding control
ns Not significant

Table A7.5.1.1-5 Effect of Fipronil on soil nitrogen content (Mean values)

		Time after initiation of test (days)				
		0	7	14	28	
						Sig
control	NH_4-N	0.70	0.21	/	/	-
	NO_2-N	0.09	0.03	0.01	0.010	-
	NO_3-N	1.19	0.95	2.10	3.18	-
	N_{min}	1.98	1.19	2.11	3.19	-
0.133mg/kg fipronil	NH_4-N	0.78	0.25	/	/	-
	NO_2-N	0.10	0.03	/	0.010	-
	NO_3-N	1.22	0.87	2.10	3.18	Ns
	N_{min}	2.10	1.15	2.10	3.19	Ns
0.667mg/kg fipronil	NH_4-N	0.62	0.21	/	/	-
	NO_2-N	0.09	0.03	/	0.017	-
	NO_3-N	1.28	0.95	2.10	3.04	Ns
	N_{min}	1.00	1.19	2.10	3.06	ns

N_{min} sum of NH_4-N, NO_2-N and NO_3-N
/ below limit of quantification (LOQ in mg/100g soil dry weight : NH_4-N= 0.17, NO_2-N = 0.07 and NO_3-N = 0.005
sig Significance
Ns Not significant

Figure A7.5.1.1-1 Effect of Fipronil on soil respiration

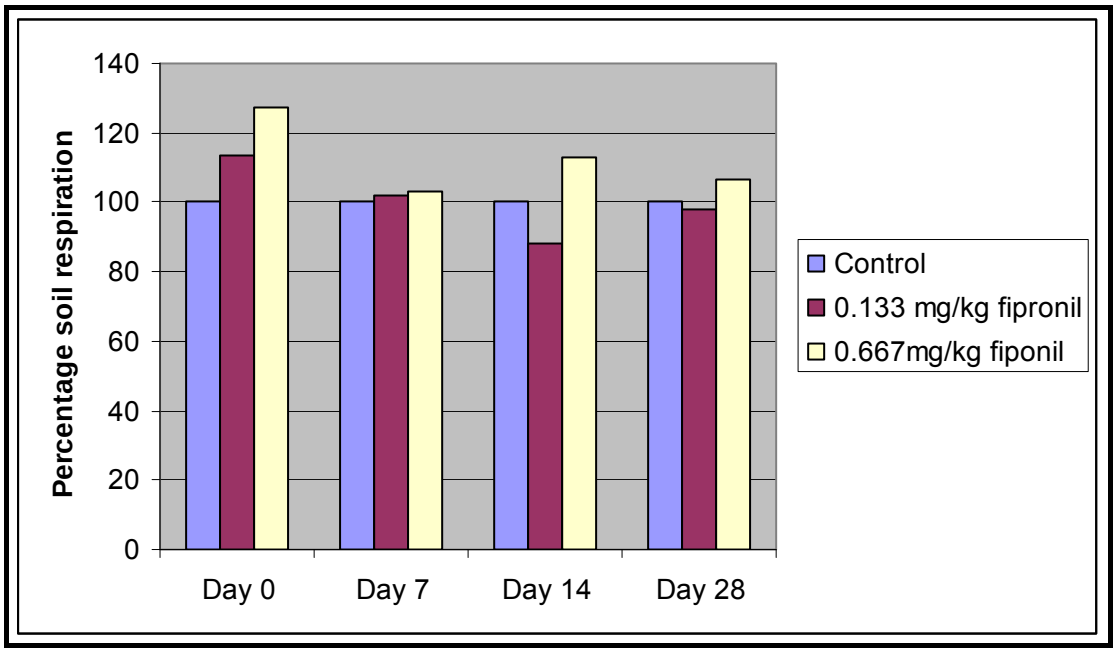
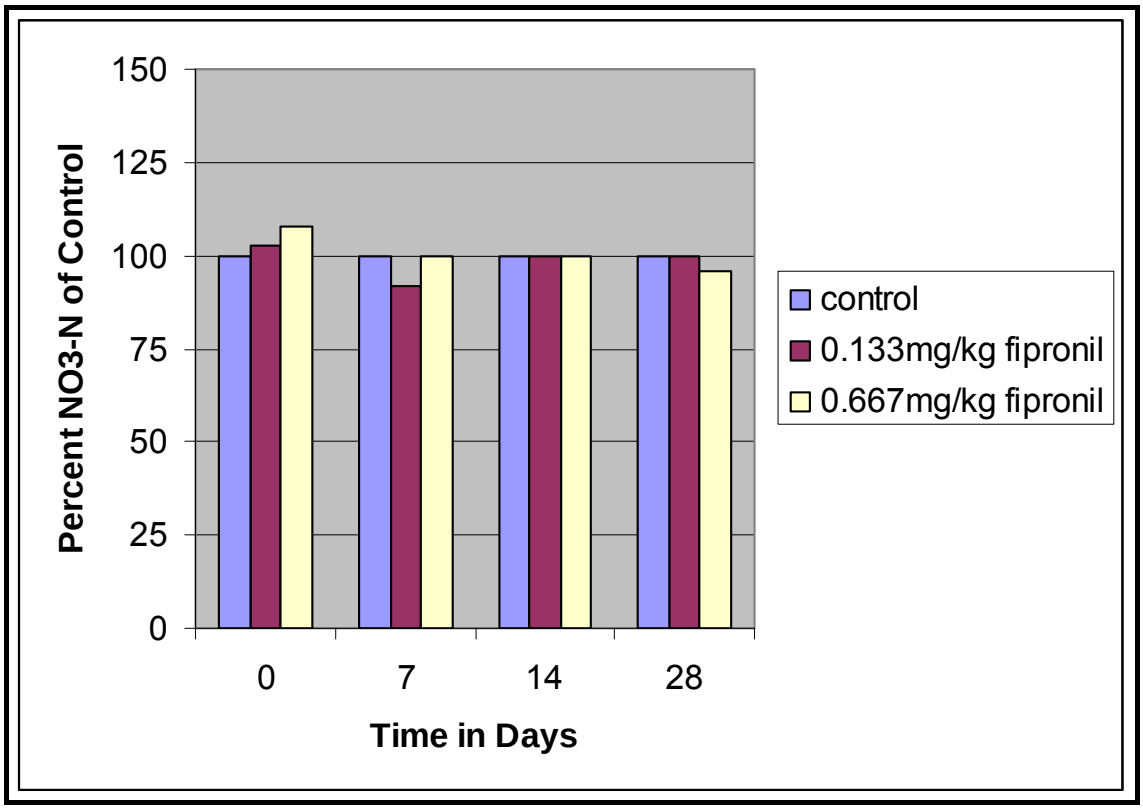


Figure A7.5.1.1-2 Effect of Fipronil on soil nitrogen content



	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 15, 2006
Materials and methods	
Conclusion	Agree with the applicant's version : no deficiencies were recorded NOEC = 0.667 mg a.s./kg dry soil
Reliability	1
Acceptability	Acceptable The test is reliable without restrictions. The results can be used in the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.1.2 **Annex Point IIIA, XIII.3.2**	**Acute toxicity test to earthworms or other soil non-target organisms**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.5.1.2/01** XXXX. The Acute Toxicity of M&B46030 to Earthworms *(Eisenia foetida)* (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD Guideline No 207 EEC Commission Directive 87/302/EEC	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**		
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	95.4% w/w	
3.1.4	Further relevant properties	None	
3.1.5	Radiolabelling	No	
3.1.6	Method of analysis	HPLC	
3.2	**Reference substance**	Chloroacetamide	
3.2.1	Method of analysis for reference substance	BDH Analar Grade	
3.3	**Testing/estimation procedure**		
3.3.1	**Test Animals**		
3.3.1.1	Species	Eisenia fetida	
3.3.1.2	Source	XXXX	
3.3.2	**Acclimitisation/ Culture**		

Section A7.5.1.2 **Annex Point IIIA, XIII.3.2**	**Acute toxicity test to earthworms or other soil non-target organisms**	
3.3.2.1 Breeding boxes	Shallow containers of approximately 20 litre volume	
3.3.2.2 Temperature	20 ± 2 °C	
3.3.2.3 Lighting	Continuous at 400 – 800 lux	
3.3.2.4 Substrate	50% by volume peat and 50% by volume of horse manure. The pH of the medium was adjusted to pH 6 – 7 by addition of calcium carbonate	
3.3.3 **Exposure**		
3.3.3.1 Duration	14 days	
3.3.3.2 Experimental Design±±	1 test concentration, 6 replicates, plus 1 control, 4 replicates. This experimental design conforms to a Limit Test to show no effect on the test animals at the maximum recommended concentration in the OECD Guidelines	
3.3.3.3 Vessels	1 litre glass beakers covered by plastic film with ventilation holes	
3.3.3.4 Loading	10 works per vessel containing 500g dry substrate	
3.3.3.5 Animals per test concentration	60	
3.3.3.6 Mean weight of worms	0.43g (SD 0.09g)	
3.3.3.7 Test concentration	1000 mg/kg	
3.3.3.8 Photoperiod	Continuous at 730 – 750 lux	
3.3.3.9 Temperature	21°C	
3.3.3.10 Renewal of test media	None	
3.3.3.11 Artificial Soil	% w/w Industrial quartz sand 69 Kaolinite clay 20 Spagnum moss peat 10 Calcium carbonate 1 (to bring pH within the range 6 ± 0.5)	
3.3.3.12 Water content	28 – 33% of dry weight of basic substrate for Day 0 and 28% on day 14	
3.3.3.13 pH	6.2 ± 0.2	
3.3.3.14 Criteria of death	Absence of reaction to physical stimulus at either end of the body	
4.1	**4. RESULTS**	
4.1.1 Fipronil	There were no significant mortalities or other adverse reactions to exposure in 60 earthworms to 1000 mg/kg fipronil for 14 days. (2/60 mortalities against 3/40 mortalities in the untreated control)	X

Section A7.5.1.2 **Annex Point IIIA, XIII.3.2**	**Acute toxicity test to earthworms or other soil non-target organisms**	
4.1.2 Reference Substance	Analysis of the mortality data obtained with chloroacetamide by the method of Thompson (1947) gave the following results LC_{50} = 15 mg/kg at 7 and 14 days with confidence limits of 14 – 17 and 14 -16 mg/kg respectively	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	An acute toxicity test was conducted over 14 days in artificial soil according to the OECD Guideline No 207. Fipronil was incorporated in artificial soil at 0 (Control) and 1000 mg/kg (dry weight). The artificial soil was prepared according to the guideline. Test conditions: temperature 21°C, continuous lighting at 730 – 750 lux, soil pH 6.1 – 6.4, soil moisture, 28 to 33% of dry weight, Test organism: *Eiseria fetida*, initial weight range 310 – 600mg/worm. Replication: 6 vessels with 10 worms each for the test item and 4 vessels with 10 worms each for the control. Observations for mortality and abnormal behaviour or appearance were made after 7 and 14 days of exposure. Worms were individually weighed prior to and after 14 days of exposure	
5.2 Results and discussion	The LC_{50} of fipronil was found to be greater than 1000 mg/kg . The No Observed Effect Concentration (NOEC) is given as > 1000mg/kg on the basis that no significant mortalities were observed after 14 days exposure. The LC_{50} of 15mg/kg at both 7 and 14 days for chloroacetamide is within the normal range found for this compound	
5.3 Conclusion		
5.3.1 Reliability	1	X
5.3.2 Deficiencies	None	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 15, 2006
Materials and methods	The applicant's version is adopted with the following revisions: 4.1.1 To be added : <u>The results are based on the nominal concentrations as the substance was not measured during the test.</u>
Conclusion	Agree with the applicant's version : no deficiencies reported
Reliability	2
Acceptability	Acceptable The test is reliable with restrictions. As the concentration of fipronil was not measured, a significant and accurate value of the NOEC cannot be given. Caution should thus be made when using the results in the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	.

Section A7.5.1.3	**Acute toxicity to plants**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.5.1.3/01** XXXX Test to determine the effects of BAS 350 00 I on seedling emergence of terrestrial plants (unpublished) XXXX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD 208 (draft)	
2.2	**GLP**	Yes	
2.3	**Deviations**	The study plan states that the daily mean temperature should be 20°C ± 6°C. This range was exceeded two times in one direction. The highest daily mean temperature was 27.4°C. This was caused by an over charge of the air conditioning system. This deviation had no negative impact on the study, because all plants were cultivated under the same conditions and the temperature was still within the range suited for the growth of the tested species.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil 80% WG	
3.1.1	Lot/Batch number	130405	
3.1.2	Specification	Nominal 80% fipronil	
3.1.3	Purity	80.3%	
3.1.4	Composition of product	Not given	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not recorded	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Not applicable the formulation is fully dispersible	
3.3	**Reference substance**	None used	

Section A7.5.1.3	**Acute toxicity to plants**	
3.3.1 Method of analysis for reference substance		
3.4 Testing procedure		
3.4.1 Dilution water	Not applicable	
3.4.2 Test plants	See Table A7.5.1.3-1	X
3.4.3 Test system	See Table A7.5.1.3-2	X
3.4.4 Test conditions	See Table A7.5.1.3-3	X
3.4.5 Test duration	See Table A7.5.1.3-3	X
3.4.6 Test parameter	Seedling emergence, phytotoxicity and plant fresh weight	
3.4.7 Sampling	See Table A7.5.1.3-3	X
3.4.8 Method of analysis of the plant material	Not applicable	
3.4.9 Quality control	Controls for each species	
3.4.10 Statistics	Calculation of mean values, standard deviation, Analysis of variance (ANOVA) followed by Dunnetts-t test ($\alpha = 5\%$), two parametric Logit-Model for EC_{25} and EC_{50} calculation	
	4. RESULTS	
4.1 Results test substance		
4.1.1 Applied initial concentration	0, 0.125, 0.5 and 2.0 mg active substance/kg dry soil	
4.1.2 Phytotoxicity rating	No phytotoxicity seen in any control or test concentration in any species	
4.1.3 Seedling emergence	No significant difference seen between controls and treatments in any species	
4.1.4 Plant Fresh weights	No significant difference seen between controls and treatments in peas, carrots or onions In corn where a significant reduction was seen in the test concentration of 0.5mg fipronil/kg dry soil. This difference was not considered as treatment related because it was not dose responsive In Oilseed rape and Oats a slight reduction (26% and 25% respectively)	
4.1.5 Root dry weights	Not measured	
4.1.6 Root length	Not measured	
4.1.7 Number of dead plants	None	
4.1.8 Effect data	None seen	
4.1.9 Concentration / response curve	Not applicable	
4.1.10 Other effects	None seen	

Section A7.5.1.3	**Acute toxicity to plants**	

4.2	**Results of controls**		
4.2.1	Number / percentage of plants showing adverse effects	None seen	
4.2.2	Nature of adverse effects	None	
4.3	**Test with reference substance**	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item : Water dispersible granule containing a nominal 80% fipronil (analysed content 80.3%). Plant Species : Pea (*Pisum sativum*); Carrot (*Daucus carota*); Oilseed rape (*Brassica napus*); Oats (*Avena sativa*); Corn (*Zea mays*); onion (*Allium cepa*). Test design : Concentration-response design, 4 variant (three test concentrations plus a control, treated with deionised water); 5 replicates per variant; 1 pot per replicate, 4 or 5 seeds per pot (species dependent); greenhouse cultivation. One day before sowing of the seeds the Test item was mixed into a defined quantity of soil substrate in the laboratory, afterwards the pots were filled with this soil substrate. After sowing of the seeds, all plants, except carrot and onion were grown for 21 days (±1 day). Carrot was grown for 25 days (±1 day). Onion was grown for 23days (±1 day). Assessments for seedling emergence and phytotoxicity were done 7, 14 and 21 days after sowing (DAS) for all plants except carrot and onion. For carrots assessments were done 11, 18 and 25 (DAS) and assessments for onion were done 9, 16 and 23 (DAS). At test termination, the plant fresh weight of the plant biomass above ground was determined. Test concentrations : 0, 0.125, 0.5 and 2.0 mg active substance/kg dry soil	
5.2	**Results and discussion**	None of the tested plant species was affected in seedling emergence. Symptoms of phytotoxicity were not found. Oilseed rape and oats showed a slight reduction in plant fresh weight at the highest tested concentration	X

Section A7.5.1.3	**Acute toxicity to plants**	
	Based on the results of this study conducted under worst case greenhouse conditions it can be concluded that pre-emergence exposure to fipronil did not result in reduced seedling emergence for all tested plant species up to the highest tested concentration of 2.0 mg fipronil/kg dry soil. For all tested plant species no symptoms of phytotoxicity were observed. A slight reduction of plant fresh weight was observed for Oilseed rape and Oats, resulting in a NOEC value of 0.5 mg fipronil/kg dry soil for these tested plant species. The NOEC value for all other tested plant species was 2.0mg fipronil/kg dry soil.	
5.2.1 EC_{20}	>2 mg fipronil/kg dry soil	X
5.2.2 EC_{50}	>2 mg fipronil/kg dry soil	
5.2.3 EC_{80}	>2 mg fipronil/kg dry soil	
5.3 Conclusion		
5.3.1 Reliability	1	X
5.3.2 Deficiencies	None	

Table A7.5.1.3-1 Test Plants

	Family	**Species**	**Common name**
Dicotyledonae	Pisum	sativum	Pea
	Daucus	Carota	Carrot
	Brassica	Napus	Oilseed rape
Monocotyledonae	Avena	sativa	Oats
	Zea	mays	Corn
	Allium	cepa	onion

Table A7.5.1.3-2 Test System

Criteria	Details
Test type	Germination
Container type	Plant pot
Seed germination potential	Controls for each species
Identification of the plant species	No
Number of replicates	5 per concentration
Numbers of plants per replicate dose	4 pea and corn 5 carrot, oilseed rape, oats and onion
Date of planting	Not applicable
Plant density	Not applicable
Date of test substance application	One day prior to sowing
Height of plants at application	Not applicable
Date of emergence, phytotoxicity rating and harvest	oilseed rape, oats pea and corn 7, 14 and 21 (±1 day) days after sowing carrot 11, 18 and 25 (±1 day) days after sowing onion 9, 16 and 23 (±1 day) days after sowing
Dates of analysis	Not applicable

Table A7.5.1.3-3 Test Conditions

Criteria	Details
Test type	Inhibition of germination
Method of application	Soil incorporation
Application levels	Not applicable
Dose rates	0, 0.125, 0.5 and 2.0 mg active substance/kg dry soil
Substrate characteristics	Natural soil, classified as a loamy sand; steam sterilized
Watering of the plants	Water content of the soil was ca 50% at time of sowing Surface watering on the day of sowing. Afterwards bottom watering according to consumption
Temperature	Average mean temperatures between 22 and 23°C
Thermoperiod	None
Light regime	16 hours light, 8 hours dark (additional light when daylight was less than 5klx
Relative humidity	Average mean RHs between 56 and 67%
Wind volatility	Not applicable – greenhouse test
Observation periods and duration of test	oilseed rape, oats pea and corn 7, 14 and 21 days after sowing carrot 11, 18 and 25 days after sowing onion 9, 16 and 23 days after sowing
Pest control	None
Any other treatments and procedures	None of the seeds was dressed

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 22, 2006
Materials and methods	The applicant's version is adopted with the following revisions: 3.4.2 Test plants The following paragraph should be added: Three dicotyledonous (*Pisum sativum, Daucus carota, Brassica napus*) and three monocotyledonous (*Avena sativa, Zea Mays and Allium cepa*) plants species were used as test organisms (See Table A7.5.1.3-1) 3.4.3 Test system The following paragraphs should be added: The seeds were placed into plastic pots approximately 8 cm in diameter with bottom watering from a reservoir in a greenhouse. The pots were filled with approximately 170 g or 180 g of fresh soil. The soil is a Loamy sand with the following characteristics : • Clay (<2µm) : 13.6% • Silt (2- 63 µm): 31.2% • Sand (> 63 µm): 55.2% • PH: 7.6% • Organic carbon: 1.2% 3.4.4 Test conditions The following paragraphs should be added One day prior to the sowing of the seeds, the pots were filled with soil substrate, into which the test item had been mixed. The water content of the soil substrate after application was 52 % of the maximum WHC max. For each plant species three test concentrations and a water treated control were tested in 5 replicates. The number of seeds placed in each replicate was 4 or 5 depending on the species. After sowing the seeds, the plants were cultivated. At day 7, 14 and 21 after sowing, the number of seedlings and the phytotoxicity was assessed. If on day 7, the rate of total seedling emergence in the control is less than 50%, the assessment was continued daily until 50% seedling emergence was reached. From this day, the plants were cultivated further for 14 days. At test termination, the plant fresh weight was determined. During the test, a light regime of 16 hours of light and 8 hours of dark was applied. Surface watering was done on the day of sowing of the seeds. Afterwards bottom watering was done according to consumption. Fertiliser was added once a week (hydroponic cilture Flory 9 Hydro) Temperature and air humidity were measured continuously. 3.4.5 Test concentrations The following paragraph should be added : 0, 0.125, 0.5 and 2.0 mg active substance/kg dry soil /5 replicates per concentration 3.4.6 Test duration and sampling during the experiment The following paragraphs should be added:

	The number of seedlings and the phytotoxicity were assessed after 7, 14 and 21 days for all plants with exception of onion and carrot. For onion the number of seedlings and phytotoxicity were assessed on day 9, 16 and 23 after sowing of the seeds. For carrot, the number of seedlings and phytotoxicity were assessed on day 11, 18 and 25 after sowing of the seeds. At test termination, the plants were directly cut ubove the ground and the plant fresh weight per replicate was determined not later than 15 minutes after cutting.
Results and discussion	The applicant's version is adopted with the following revisions …"***A slight reduction of plant fresh weight was observed for Oilseed rape and Oats, resulting in a NOEC value of 0.5 mg fipronil/kg dry soil for these tested plant species. The NOEC value for all other tested plant species was 2.0mg fipronil/kg dry soil***. The results are based on the nominal concentrations as the substance was not measured during the test." 5.2.1 **<Comment>** : It is stated that the EC_{20} >2 mg fipronil/kg dry soil. This cannot be the case as the EC25 for Oilseed rape is 1.9 mg fipronil/kg dry soil.
Conclusion	NOEC = 0.5 mg a.s./kg dry soil
Reliability	2
Acceptability	Acceptable The test is reliable with restrictions. As the concentration of fipronil was not measured, a significant and accurate value of the NOEC cannot be given. Caution should thus be made when using the results in the risk assessment.
Remarks	

	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.2	**Terrestrial tests, long-term tests**

Section A7.5.2.1 **Annex Point IIIA, XIII.3.2**	**Reproduction study with other soil non-target macro-organisms**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.5.2.1/01** XXXX Effects on reproduction and growth of earthworms (*Eisenia andrei*) in artificial soil (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes ISO 11268 part II (draft), BBA VI-2-2 (1994°	
2.2	**GLP**	Yes	
2.3	**Deviations**	1. For technical reasons, the % humidity of the substrate was calculated according to the dry weight of the sediment, instead of according to its water holding capacity as mentioned in the guideline. However, no standing water or free water appeared in the test vessels. 2. At test termination, temperature of the water bath used for extraction of the juvenile worms was approximately 90°C instead of 50 to 60°C as given in the protocol. Therefore, the juvenile worms appeared at the substrate surface before 60 minutes specified in the protocol . 3. Due to a technical problem, the temperature in the test enclosure exceeded the recommended range of 20±2°C during a period of approximately 2 days (Days 44 and 45) during the test period. This was not considered to have had any significant effect of the results of the study. It was the opinion of the performing laboratory that these deviations did not affect the results of the study.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil	
3.1.1	Lot/Batch number	93, 148/6123.B	
3.1.2	Specification	As given in Section 2	

Section A7.5.2.1 **Annex Point IIIA, XIII.3.2**		**Reproduction study with other soil non-target macro-organisms**	
3.1.3	Purity	960g/kg	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not recorded	
3.2	**Reference substance**	Carbendazim	
3.2.1	Method of analysis for reference substance	Not recorded	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	None	
3.3.2	Application of the test substance	Dry admixture to the artificial soil	
3.3.3	Test organisms	As given in Table A7.5.2.1-1	
3.3.4	Test system	As given in Table A7.5.2.1-2	
3.3.5	Test conditions	As given in Table A7.5.2.1-3	
3.3.6	Test duration	Mortality adult worms – 4 weeks Survival offspring – 8 weeks	
3.3.7	Test parameter	Mortality adult worms and survival offspring	
3.3.8	Examination	Observation (and weighing of adults)	
3.3.9	Monitoring of TS concentration	No analytical verification was performed. Results are presented in terms of nominal concentrations	
3.3.10	Statistics	Comparison of control groups with toxic standard : F test Comparison of treated groups to the control group: Bartlett test	
		4. RESULTS	
4.1	**Filter paper test**	Not applicable	
4.1.1	Concentration		
4.1.2	Number / percentage of animals showing adverse effects		
4.1.3	Nature of adverse effects		
4.2	**Soil test**		
4.2.1	Initial concentrations of test substance	No analytical verification was performed. Results are presented in terms of nominal concentrations i.e 63,125, 250, 500 and 1000mg/kg soil	
4.2.2	Effect data (mortality)	See Table A7.5.2.1-4	X

Section A7.5.2.1 **Annex Point IIIA, XIII.3.2**		**Reproduction study with other soil non-target macro-organisms**	
4.2.3	Concentration / effect curve	Not applicable	
4.2.4	Other effects	See Table A7.5.2.1-4	
4.3	**Results of controls**		
4.3.1	Mortality	See Table A7.5.2.1-4	
4.3.2	Number / percentage of earthworms showing adverse effects	See Table A7.5.2.1-4	
4.3.3	Nature of adverse effects	See Table A7.5.2.1-4	
4.4	**Test with reference substance**		
4.4.1	Concentrations	3mg/kg	
4.4.2	Results	See Table A7.5.2.1-4	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test substance: fipronil (synthesis batch 97.148/6123B) certified purity 960g/kg incorporated in artificial soil at 0 (control) 63, 125, 250, 500 and 1000 mg/kg soil (dry weight). A toxic standard (carbendazim at 3 mg/kg) was run in parallel. The artificial soil was prepared according to OECD guideline 207 and received additionally 10g of dried horse manure per 500g soil. **Test Conditions** Temperature: 20±2°C Lighting: Continuous, at 400 to 800 lux pH range: 5.6 – 5.7 soil moisture: approximately 60% of dry weight **Test Organism** adult clitellated earthworms *Eisenia foetida andrei*, at least 2 months of age, mean weight range 250 – 600mg at test initiation. **Replication**: 4 vessels with 10 worms each per test item concentration and the control. **Test procedure** Finely ground cattle manure was provided as food according to the guideline. The exposure duration was 8 weeks and was divided in two parts which were as follows: Part 1: The test was performed by exposing seven groups of 40 adult earthworms to five concentrations of the test substance in artificial substrate, one artificial substrate control and one artificial substrate toxic reference for a period of 4 weeks. A mortality count was performed at Day 28 after which the adult earthworms were removed and weighed.	

Section A7.5.2.1 **Annex Point IIIA, XIII.3.2**	**Reproduction study with other soil non-target macro-organisms**	
	Part 2: at the end of the period of 4 weeks, the soil substrate was refilled to the test containers and incubated for another period of 4 weeks under test condition to allow offspring to develop from the cocoons. The number of offspring produced was determined and compared to the control with the help of the Dunnett-test (toxic standard: t-test).	
5.2 Results and discussion	No adverse effects on survival, growth or reproduction were observed even at the highest concentration tested. Food consumption was comparable between the different test item concentrations and the controls. Thus the NOEC from this study was 1000 mg/kg soil the highest concentration tested.	X
5.3 Conclusion		
5.3.1 Other conclusions		
5.3.2 Reliability	1	X
5.3.3 Deficiencies	None	

Table A7.5.2.1-1 Test organisms

Criteria	**Details**
Species / strain	*Eisenia andrei*
Source of the initial stock	XXXX
Culturing techniques	Not recorded
Age / weight	Age: at least 2months Weight: 250 – 600g
Pretreatment	Maintained under test conditions for 3 – 4 weeks prior to treatment

Table A7.5.~~1.2~~ 2.1-2 Test system X

Criteria	Details
Artificial soil test substance	Artificial Soil as specified in OECD 207 with the addition of dried horse manure at 10g per 500g as food
Test mixture	The test material was mixed directly into the artificial soil
Size, volume and material of test container	1.5 litre disposable plastic containers covered with a plastic lid and perforated with ventilation holes
Amount of artificial soil (kg)/container	500g dry artificial substrate humidified with approximately 300ml deionized water
Nominal levels of test concentrations	63, 125, 250, 500 and 1000
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Not recorded
Test performed in closed vessels due to significant volatility of test substrate	Not applicable

Table A7.5. .~~1.2~~ 2.1-3 Test conditions X

Criteria	Details
Test temperature	20 ± 2°C
Moisture content	50 –55%
pH	5.6 – 5.7
Adjustment of pH	No
Light intensity / photoperiod	400 – 800 lux/16 hours light/8 hours dark
Relevant degradation products	Not relevant

Table A7.5. .~~1.2~~ 2.1-4 Mean Mortality and effect data X

	Control	Toxic Control	62.5 mg/kg fipronil	125 mg/kg fipronil	250 mg/kg fipronil	500 mg/kg fipronil	1000 mg/kg fipronil
Mortality	0	0	0	2.5	0	0	0
Growth (%, ±SD	36±6	54±9	35±5	43±7	38±12	50±6	53±9
Mean no of offspring per vessel, ±SD	111±7	37±11	101±8	107±11	96±24	79±13	90±22

Fipronil concentrations are nominal

X

Table A7.5. ~~1.2~~ 2.1-5 Validity Criteria for Acute Earthworm Test According to OECD Guideline 207

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	✓	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 16,2006
Materials and methods	
Results and discussion	Agree with the applicant's version with the following amendements : 5.2 *No adverse effects on survival, growth or reproduction were observed even at the highest concentration tested. Food consumption was comparable between the different test item concentrations and the controls. Thus the NOEC from this study was ≥1000 mg/kg soil* which *is the highest concentration tested.* **<Comment>** : No statistically significant effects were observed on mortality, growth and reproduction. Nevertheless, mean growth of the individuals in the highest treatment was higher than in the control after 28 days.
Conclusion	Agree with the applicant's version.
Reliability	2
Acceptability	Acceptable The test is reliable with restrictions. As the concentration of fipronil was not measured, a significant and accurate value of the NOEC cannot be given. Caution should thus be made when using th results in the risk assessment.
Remarks	Errors in titles of tables were corrected in bold and underlined.
	COMMENTS FROM …
Date	.
Conclusion	
Reliability	
Acceptability	
Remarks	.

Section A7.5.2.2 **Annex Point IIIA, XIII 3.2**	**Long term test with terrestrial plant**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Please see: "General comment on studies summarized in this section" A7.5	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and methods	
Conclusion	
Reliability	
Acceptability	Agree with the applicant justification.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.3 **Annex Point IIIA, XIII.1**	**Effects on birds**

Section A7.5.3.1.1 **Annex Point IIIA, XIII.1.1**	**Acute oral toxicity to bobwhite quail**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A7.5.3.1.1/01** XXXX (1990) M&B 46030 technical: 21day acute oral LD50 study in bobwhite quail *(Colinius viginanus)* XXXX (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA FIFRA E, 71-1	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil, technical	
3.1.1	Lot/Batch number	JJW-2127	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	>95% w/w	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not applicable	
3.2	**Administration of the test substance**	Gelatine capsules	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		

Section A7.5.3.1.1 **Annex Point IIIA, XIII.1.1**	**Acute oral toxicity to bobwhite quail**	
3.4.1 Test organisms	See Table A7.5.3.1.1.1-2	
3.4.2 Test system	See Table A7.5.3.1.1.1-3	
3.4.3 Diet		
3.4.4 Test conditions	See Table A7.5.3.1.1.1-4	
3.4.5 Duration of the test	21 days	
3.4.6 Test parameter	Mortality (bodyweight, food consumption, behaviour and gross macroscopy.)	X
3.4.7 Examination / Observation	Mortality, behaviour were observed daily bodyweight and food consumption were measured daily gross macroscopy was performed at test termination	
3.4.8 Statistics	Data on mortality and body weight were statistically evaluated based on regression analysis and analysis of variance, respectively	
	4. RESULTS	
4.1 Limit test / Range finding test	Not applicable	
4.1.1 Concentration		
4.1.2 Number / percentage of animals showing adverse effects		
4.1.3 Nature of adverse effects		
4.2 Results test substance		
4.2.1 Applied concentrations	Nominal values	
4.2.2 Effect data (mortality)	See table 7.5.3.1.1.1-5	
4.2.3 Body weight	See table 7.5.3.1.1.1-6	
4.2.4 Feed consumption	See table 7.5.3.1.1.1-7	
4.2.5 Concentration / response curve	Not applicable	
4.2.6 Other effects	Behaviour No abnormal behavioural reactions or systemic signs of toxicity were noted in the vehicle control or 1 mg/kg bw groups. Signs of toxicity in the remaining treatment groups included lethargy, head bobbing when disturbed, chalky diarrhoea, anorexia, stumbling ataxia, tremors, tachypnea, wing beat convulsions, tetany, spasms, loss of balance, piloerection, sitting, failure to respond to external stimuli, gasping for breath, noticeable weight loss, the appearance of weakness or listlessness and death. Total remission was achieved by surviving birds by test day 18.	

Section A7.5.3.1.1 **Annex Point IIIA, XIII.1.1**	**Acute oral toxicity to bobwhite quail**	

		Gross macroscopy Total gross pathology findings in birds that died during the study included abnormalities in the digestive system or friable livers. No such findings were reported from the gross pathological examination from 21-day surviving bird, with the exception of one female with an enlarged liver. No behavioural abnormalities or pathological findings were reported from the controls and the lowest dose groups of 1 mg/kg bw.	X
4.3	**Results of controls**		
4.3.1	Number / percentage of animals showing adverse effects	Considered together with results for test substance	
4.3.2	Nature of adverse effects	Considered together with results for test substance	
4.4	**Test with reference substance**	None	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: Fipronil technical, purity >95% : administered in gelatine capsules (carrier acetone allowed to evaporate prior to administration). Test organism: Bobwhite Quail *(Colinus virginianus),* 43 – 44 weeks of age; initial mean bodyweight 215 (180 – 251) g/bird, fasted approximately 18 hours prior to dosing. Test groups: vehicle control plus 5 test item groups dosed at 1, 4.64, 10, 21.5 or 46.4 mg ai/kg bw (defined based on preliminary testing). Each group consisted of 5 male and 5 females in a single pen. Daily observations were made over 21 days for mortalities or clinical signs of intoxication. Individual bodyweight was determined on days 1, 3, 7, 14 and 21. Group food consumption values were recorded on test days 3, 7, 14 and 21. All birds that died during the investigation plus 2 arbitrarily selected birds among the 21 day survivors from the controls and the test groups dosed at 1, 4.64 and 10 mg/kg bw were subjected to gross pathological examinations. Data on mortality and body weight were statistically evaluated based on regression analysis and analysis of variance, respectively.	

Section A7.5.3.1.1 **Annex Point IIIA, XIII.1.1**	**Acute oral toxicity to bobwhite quail**

5.2	**Results and discussion**	Statistically significant effects on bodyweight were observed from day 3 through day 14 in birds dosed at 4.64 and 10 mg/kg but birds recovered until the end of the test. Food consumption of birds appeared temporarily reduced but was comparable to control values at the end of the test. Behavioural abnormalities reported from birds, dosed at 4.64mg/kg and above included lethargy and weakness. Total remission of symptoms in the surviving birds was noted by day 18 of the study at the latest. Total gross pathology findings in birds that died during the study included abnormalities in the digestive system or friable livers. No such findings were reported from the gross pathological examination from 21-day surviving bird, with the exception of one female with an enlarged liver. No behavioural abnormalities or pathological findings were reported from the controls and the lowest dose groups of 1 mg/kg bw. However the study director considered the NOED to be below 1 mg/kg bw, based on temporarily and slightly reduced food consumption at this level during three days after dosing (73% of control), despite the lack of statistical verification. Taking into account the absence of any further indication of toxic effects at this level (absence of mortality, bodyweight effects, clinical signs or pathological findings) setting the NOED level at 1 mg/kg bw would appear justified.
5.2.1	LD_{50}	The 21 day acute oral LD50 of fipronil in bobwhite quail was determined at 11.3 mg ai/kg bw
5.3	**Conclusion**	
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Table A7.5.3.1.1-1 Method of Administration of the Test Substance

Criteria	Details
Water	Fipronil was dissolved in acetone/water
Organic carrier	None
Concentration of the carrier	20 ng/ml
Other vehicle	Gelatine capsule
Function of the carrier / vehicle	Appropriate quantities of the test material solution were dispensed into the capsules

Table A7.5.3.1.1-2 Test Animals

Criteria	Details
Species/strain	Bobwhite Quail *(Colinus virginianus),* indistinguishable from wild birds
Source	XXXX
Age (in weeks), sex and initial body weight (bw)	Age: 36 weeks Male and females Weight: 215 (180 – 251) g/bird (at time of dosing)
Breeding population	Yes
Feed	Commercial duck food
Age at time of first dosing	43 – 44 weeks
Health condition / medication	All birds were examined prior to testing and found to be normal and active

Table A7.5.3.1.1-3 Test System

Criteria	Details
Test location	Indoors
Holding pens	24 x 21 x 15 inch wire pens over steel pans
Number of animals	10 birds (5 male and 5 female per pen)
Number of animals per pen [cm^2/bird]	Not determined
Number of animals per dose	10
Pre-treatment / acclimation	Acclimatised under test conditions
Diet during test	Commercial duck food
Dosage levels (of test substance)	1, 4.64, 10, 21.5 and 46.4 mg/kg
Replicate / dosage level	1
Dosing method	Capsule
Dosing volume per application	One capsule
Frequency, duration and method of animal monitoring after dosing	daily
Time and intervals of body weight determination	Weekly

Table A7.5.3.1.1-4 Test Conditions (housing)

Criteria	Details
Test temperature	62 – 84 °F ≡ °F
Shielding of the animals	Indoors
Ventilation	Not recorded
Relative humidity	53 – 82%
Photoperiod and lighting	Fluorescent lights on for 8 hours per day

Table A7.5.3.1.1-5 Mortality Data

Dose level mg/kg	Cumulative Mortality No and (%)			
	Test days 1 – 3	Test days 4 – 7	Test days 8 – 14	Test days 15 – 21
0	0 (0)	0 (0)	0 (0)	0 (0)
1	0 (0)	0 (0)	0 (0)	0 (0)
4.64	0 (0)	0 (0)	0 (0)	0 (0)
10	0 (0)	0 (0)	3 (30)	3 (30)
21.5	0 (0)	3 (30)	9 (90)	10 (100)
46.4	4 (40)	10 (100)	10 (100)	10 (100)

Table A7.5.3.1.1-6 Mean Bodyweight data

Dose level mg/kg	Mean bodyweight g (SD)				
	0 hour	Test day 3	Test day 7	Test day 14	Test day 21
0	219 (±13)	219 (±17)	219 (±20)	223 (±22)	224 (±23)
1	214 (±14)	208 (±10)	208 (±8)	211 (±6)	215 (±6)
4.64	212 (±22)	193** (±19)	193** (±19)	194** (±18)	204 (±17)
10	215 (±20)	199** (±18)	181** (±19)	185* (±29)	205 (±25)
21.5	219 (±15)	196* (±17)	189** (±26)	A	-
46.4	212 (±18)	186** (±15)	-	-	-

- indicates that there were no survivors in this group during this interval
A There was only one survivor in this group during this interval
* A statistical significant difference exists at the 95% confidence level
** A statistical significant difference exists at the 99% confidence level

Table A7.5.3.1.1-7 Mean Estimated food consumption

Dose level mg/kg	Mean estimated food consumption per bird per day g			
	Test days 1 – 3	Test days 4 – 7	Test days 8 – 14	Test days 15 – 21
	15	15	21	15
1	11	14	22	14
4.64	8	11	23	15
10	3	2	12	22
21.5	3	1	0	-
46.4	2	-	-	-

- indicates that there were no survivors in this group during this interval

Table A7.5.3.1.1-8 Validity Criteria for Avian Acute Oral Toxicity According to EPA OPPTS 850.2100

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	✓	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4 May 2007
Materials and methods	**3.4.6 Test parameter** *Mortality (bodyweight, food consumption, behaviour and gross macroscopy.*) pathology **4.2.6 Other effects** *Gross ~~macroscopy~~* pathology
Conclusion	Agree with th applicant
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.3.1.2 **Annex Point IIIA, XIII.1.2**	**Short term toxicity in bobwhite quail**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A.7.5.3.1.2/01** XXXX. M&B46030 technical: 22 day Acute Dietary LC_{50} Study in Bobwhite Quail XXXX (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA FIFRA E 71-2	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Technical fipronil	
3.1.1	Lot/Batch number	JJW-2092/1	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	>95% w/w	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	HPLC with UV detection	
3.2	**Administration of the test substance**	*Via* the diet	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	See Table A7.5.3.1.2.1-2	
3.4.2	Test system	See Table A7.5.3.1.2.1-3	

Section A7.5.3.1.2 Annex Point IIIA, XIII.1.2	**Short term toxicity in bobwhite quail**	
3.4.3 Diet	Commercial bird food – Purina Game Bird Startena	
3.4.4 Test conditions	See Table A7.5.3.1.2.1-4	X
3.4.5 Duration of the test	22 days (5 days treatment and 17 days post treatment)	
3.4.6 Test parameter	Mortality, behaviour, clinical signs, food consumption bodyweight and gross macroscopy	X
3.4.7 Examination / Observation	Daily	
3.4.8 Statistics	The LC_{50} was based on regression analysis	
	4. RESULTS	
4.1 Limit test / Range finding test		
4.1.1 Concentration		
4.1.2 Number / percentage of animals showing adverse effects		
4.1.3 Nature of adverse effects		
4.2 Results test substance		
4.2.1 Applied concentrations	See Table A7.5.3.1.2.1-5	
4.2.2 Effect data (mortality)	See Table A7.5.3.1.2.1-6	
4.2.3 Body weight	See Table A7.5.3.1.2.1-7	
4.2.4 Feed consumption	See Table A7.5.3.1.2.1-8	
4.2.5 Concentration / response curve	Not applicable	
4.2.6 Other effects	**Clinical signs** No clinical signs of toxicity were noted in the vehicle control groups or in the 4.9, 9.8 and 19.5 mg/kg diet groups. In the higher concentration groups lethargy, white coloured diarrhoea and anorexia were noted. Total remission of all clinical signs was achieved in survivors by the end of test day 6. **Gross pathology** Gross pathological examinations of the thirty-two birds that died during the investigation and of twenty selected survivors at termination revealed no abnormal pathological findings.	
4.3 Results of controls		
4.3.1 Number / percentage of animals showing adverse effects	None	

Section A7.5.3.1.2 **Annex Point IIIA, XIII.1.2**	**Short term toxicity in bobwhite quail**

4.3.2	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**	None	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: Fipronil technical, >95% purity mixed into a standard bird diet in acetone vehicle. Homogeneity, stability and accuracy of the dietary concentrations were analytically confirmed. Test organisms: 14 day old Bobwhite quail (*colinus virginianus*), obtained from a commercial supplier, initial mean bodyweights 26 - 30g/bird in the controls and 27 – 31g/bird in the test item treated groups. Test groups consisted of 10 birds housed together. Over 5 consecutive days, test diets were fed to 5 vehicle control groups (0 mg/kg diet) and to 7 test groups at dietary concentrations of 4.9, 9.8, 19.5, 39, 156, 312 and 625 mg/kg diet. From day 6 to day 22 all surviving birds were offered untreated diet. Daily observations were made over 22 days for mortality and other signs of intoxication. Group mean bodyweight was determined at test initiation and test termination. Food consumption was estimated over 4 or 5 day periods. Gross pathological examinations were performed on all 32 bird that died during the investigation and on 4 arbitrarily selected birds from the controls and the test groups with surviving birds. The LC_{50} was based on regression analysis.	
5.2	**Results and discussion**	No mortalities, signs of intoxication or effects on food consumption and bodyweight were reported from the controls or the test groups of 4.9, 9.8 or 19.5 mg/kg diet. The 5 day LC50 of fipronil technical in Bobwhite quail was calculated to be 48 mg/kg diet, with 95% confidence intervals of 38.7 and 59.5 mg/kg diet. At 39 mg/kg diet, clearly reduced food consumption (3g/bird/day vs 6-7g/bird/day in the controls or lower dose levels) was observed during the exposure period. Consequently the final body weight of these birds was lower than that of the controls. Mortality and clinical signs of toxicity were noted in birds offered 156 mg/kg diet and above. All surviving birds recovered one day after termination of exposure. Gross pathological examination of the thirty-two birds that died during the investigation and the twenty selected survivors at termination revealed no abnormal pathological findings. Based on all parameters observed, the NOEL was determined at 19.5 mg/kg diet	

Section A7.5.3.1.2 Short term toxicity in bobwhite quail **Annex Point IIIA, XIII.1.2**		
5.2.1 NOEL	19.5 mg/kg diet	
5.2.2 LC_{50}	48.0 mg/kg diet (with 95% confidence limits of 38.7 59.5)	
5.3 Conclusion		
5.3.1 Reliability	1	X
5.3.2 Deficiencies	None	X

Table A7.5.3.1.2.1-1 Method of Administration of the Test Substance

Criteria	Details
Solvent	Acetone
Organic carrier	Standard laboratory bird diet
Concentration of the carrier [mg/kg]	5000 (then further diluted with diet to give the remaining dose levels

Table A7.5.3.1.2.1-2 Test Animals

Criteria	Details
Species/strain	Bobwhite quail (*Colinus virginianus*) phenotypically indistinguishable from wild birds
Source	XXXX
Age, sex and initial body weight (bw)	14 days of age, males and females, bodyweight
Age range within the test	14 – 36 days
Breeding population	No
Age at time of first dosing	14 days
Health condition / medication	Only healthy birds were selected for the study

Table A7.5.3.1.2.1-3 Test System

Criteria	Details
Test location	Indoors
Holding pens	Wire pens 45.7 x 61 x 45.7 cm
Number of animals	10 per pen
Number of animals per dose	10 (50 for the controls)
Pre-treatment / acclimation	14 days acclimatisation
Diet during test	Standard laboratory bird diet
Dosage levels (of test substance)	0 (solvent control) 4.9, 9.8, 19.5, 39, 156, 312 and 625 mg/kg diet.
Replicate / dosage level	One (5 for controls)
Dosing method	Dietary
Dosing volume per application	*Ad libitum*
Frequency, duration and method of animal monitoring after dosing	Daily
Time and intervals of body weight determination	Study initiation and termination

Table A7.5.3.1.2.1-4 Test Conditions (housing)

Criteria	Details
Test temperature	92 – 114°F
Shielding of the animals	Indoors
Ventilation	Not recorded
Relative humidity	25 – 58%
Photoperiod and lighting	Fluorescent lights on 24 hours a day

Table A7.5.3.1.2.1-5 Measured Dietary Concentrations

Nominal concentration (ppm)	Measured concentration (ppm)	Recovery (%)
Homogeneity and dose verification data		
0	<1.0	N/A
4.9 (top)	4.00	81.6
4.9 (middle)	3.48	71.0
4.9 (bottom)	4.43	90.4
19.5	16.8	86.2
39	35.5	91.0
156	140	89.7
312	257	82.4
Stability data*		
0	<1.0	N/A
4.9	5.23	107
Recovery data		
4.83	3.82	79.1
308	296	96.1

Table A7.5.3.1.2.1-6 Mortalities recorded during the study

mg/kg diet	Mortalities			
	Day			
	3	4	5	22 (cumulative)
0	0	0	0	0/10
4.9	0	0	0	0/10
9.8	0	0	0	0/10
19.5	0	0	0	0/10
39	0	0	0	2/10
156	0	2	0	10/10
312	3	5	2	10/10
625	2	5	2	10/10

Table A7.5.3.1.2.1-7 Mean Bodyweight

Nominal concentration (ppm)	Average bodyweight (g)	
	0 hour	Day 22
Control Group 1	26	105
Control Group 2	30	107
Control Group 3	27	99
Control Group 4	29	105
Control Group 5	28	105
4.9	30	105
9.8	30	101
19.5	31	86
39	27	-
156	29	-
312	27	-
625	28	-

- Indicates that there were no survivors in this goup at the end of this interval

Table A7.5.3.1.2.1-9Estimated food consumption

Nominal Concentration	Total Estimated Food Consumption (g)					Estimated Food consumption per bird per day (g)				
	Day 0-5	Day 6-10	Day 11-15	Day 16-20	Day 21-22	Day 0-5	Day 6-10	Day 11-15	Day 16-20	Day 21-22
Control Group 1	335	452	481	715	298	7	9	10	14	15
Control Group 2	34.3	461	452	607	248	7	9	10	12	12
Control Group 3	316	425	445	613	215	8	9	9	12	11
Control Group 4	312	439	475	668	262	8	9	10	14	13
Control Group 5	327	449	445	609	246	7	9	9	12	12
4.9	346	502	479	682	263	7	10	10	14	13
9.8	309	437	440	519	242	6	9	9	10	12
19.5	302	474	480	557	245	6	9	10	11	12
39	143	325	366	400	194	3	8	9	10	12
156	54	-	-	-	-	1	-	-	-	-
312	-	-	-	-	-	-	-	-	-	-
625	-	-	-	-	-	-	-	-	-	-

Table A7.5.3.1.2.1-6 Validity Criteria for Short-Term Toxicity Test According to OECD 205

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	✓	
Test substance concentration > 80% of nominal concentration throughout the dosing period	✓	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	✓	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	03 May 2007
Materials and methods	**3.4.4 Test conditions** According to the OECD guideline, relative humidity for this species should be be between 50 to 70% **3.4.6 Test parameter** *Mortality, behaviour, clinical signs, food consumption bodyweight and ~~gross macroscopy~~* gross pathology **5.3.2 Deficiencies** Relative humidity values are low (25 to 58%) compared to the recommanded values
Conclusion	Agree with the applicant
Reliability	2 As the results of relative humidity are below the recommanded values, the test is considered acceptable with restrictions
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.3.1.3 **Annex Point IIIA, XIII.1.3**	**Effects on reproduction– bobwhite quail**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.5.3.1.3/01** XXXX. M&B46030 technical: Toxicity and reproduction study in bobwhite quail XXXX (unpublished) XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes	
2.2	**GLP**	USEPA FIFRA E, 71-4	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Technical fipronil	
3.1.1	Lot/Batch number	78GC90	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	96.7% w/w	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	HLPC with UV detection	
3.2	**Administration of the test substance**	Table A7.5.3.1.3-1	
3.3	**Testing procedure**		
3.3.1	Test organisms	Table A7.5.3.1.3-2	
3.3.2	Test system	Table A7.5.3.1.3-3	
3.3.3	Diet		
3.3.4	Test conditions	Table A7.5.3.1.3-4	X
3.3.5	Duration of the test	142 days of test substance administration	

Section A7.5.3.1.3 **Annex Point IIIA, XIII.1.3**	**Effects on reproduction– bobwhite quail**	
3.3.6 Test parameter	Mortality, clinical signs, food consumption, body weight, egg thickness, reproductive success, F1- generation body weight	X
3.3.7 Examination / Observation	Observations taken daily; bodyweight every two weeks and food consumption bi-weekly	
3.3.8 Statistics	The NOEC determination included data analysis with ANOVA and contingency table analysis	
	4. RESULTS	
4.1 Limit test / Range finding test	No	
4.1.1 Concentration	Not applicable	
4.1.2 Number / percentage of animals showing adverse effects	Not applicable	
4.1.3 Nature of adverse effects	Not applicable	
4.2 Results test substance		
4.2.1 Applied concentrations	Analysis showed that measured dietary concentration of fipronil in avian diet used in this study was between 41 and 118% of nominal	X
4.2.2 Effect data (mortality and reproductivity)	See Table A7.5.3.1.3-5	
4.2.3 Body weight	See Table A7.5.3.1.3-5	
4.2.4 Feed consumption	See Table A7.5.3.1.3-5	
4.2.5 Results of residue analysis	See Table A7.5.3.1.3-5	
4.2.6 Other effects	See Table A7.5.3.1.3-5	
4.3 Results of controls		X
4.3.1 Number / percentage of animals showing adverse effects	None	
4.3.2 Nature of adverse effects	Not applicable	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Test item Fipronil technical (96.7% purity), mixed into standard bird diet (vehicle acetone). Homogeneity stability and accuracy of the dietary concentrations were analytically confirmed.	

Section A7.5.3.1.3 **Annex Point IIIA, XIII.1.3**	**Effects on reproduction– bobwhite quail**	
	Test organism Bobwhite quail (*Colinus virginianus*), 26 weeks of age at exposure, initiation and approaching their first breeding season; initial mean bodyweight approx 205 – 225 g/bird. Birds were offered the test item over 142 days at dietary concentrations of 0 (vehicle control), 0.2, 2 and 10 mg/kg diet. The dose levels were based on the results of a 28 day pilot study. The control and each test item group consisted of 12 pens with 1 male and 2 female each. Eggs laid by the parental generation were hatched in an incubator system. Hatchings from all test groups were observed and offered untreated diet over 14 days. Daily observations were made during the test of the parental generation for mortality and other signs of toxicity. Individual bodyweight was determined at test initiation, after 2, 4, 6 and 8 weeks and termination of the study. Average feed consumption was estimated by pen and reported on a bi-weekly basis. Eggs laid were counted and assessed for quality parameter (size, shell thickness, egg damages, egg fertility and embryo development). Gross necropsy was performed on all adult birds that died or were sacrificed during the course of the test and on 50% of all adult birds surviving until test termination. F1 generation birds were observed daily for 2 weeks for mortality or signs of toxicity. Individual bodyweight was determined on days 1 and 14. Gross necropsy was performed on chicks found dead during this 2-week period and on selected survivors. The NOEC determination included data analysis with ANOVA and contingency table analysis.	
5.2 Results and discussion	No adverse effects were observed during the test that were attributable to the test item. Mortality, food consumption and bodyweight of the parental generation, their reproductive success and the viability of the offspring were comparable in the control and the test item treated groups. A few measurements taken at individual time points during the study resulted in temporary statistically significant differences (i.e. proportion of cracked eggs, hatchling bodyweight) but these differences were small and not consistent in time or lacked dose response. They were therefore attributed to normal biological variation rather than to exposure to the test item. Based on nominal but analytically confirmed concentrations and on mean bodyweight and food consumption values reported for the first 8 weeks and the last 2 weeks of the test the NOEC of 10 mg/kg diet is corresponding to a daily uptake of approximately 0.8mg/kg bodyweight per day.	
5.2.1 NOEC	10 mg/kg diet	
5.3 Conclusion		
5.3.1 Reliability	1	

Section A7.5.3.1.3 Effects on reproduction– bobwhite quail **Annex Point IIIA, XIII.1.3**		
5.3.2 Deficiencies	none	X

Table A7.5.3.1.3-1 Method of Administration of the Test Substance

Criteria	**Details**
Solvent	Acetone
Carrier	Commercial game bird feed
Mixing	Hobart H-600DT mixer

Table A7.5.3.1.3-2 Test Animals

Criteria	**Details**
Species/strain	Bobwhite quail (*Colinus virginianus*) phenotypically indistinguishable from wild birds
Source	XXXX
Age (in weeks), sex and initial body weight (bw)	21 weeks of age male and female initial bodyweight 205 – 225g per bird
Age range within the test	25 – 33 weeks
Breeding population	yes
Amount of food	*Ad libitum*
Age at time of first dosing	25 weeks
Health condition / medication	Only healthy birds were selected for the study
Pre-treatment	4 weeks acclimatisation

Table A7.5.3.1.3-3 Test System

Criteria	Details
Test location	Indoors
Holding pens	53.3 x 61.0 x 38.1 cm steel wire pens constructed of 1 inch wire mesh maintained over galanised dropping pens
Number of animals (male/female)	One male and two females
Number of animals per pen [cm^2/bird]	Not recorded
Number of animals per dose	36 (12 x 3)
Pre-treatment / acclimation	Acclimatised for 4 weeks
Diet during test	Commercial avian diet containing the appropriate amount of test substance
Dosage levels (of test substance)	0, 0.2, 2.0 and 10 mg/kg diet
Replicate / dosage level	12
Dosing method	Dietary administration
Dosing volume per application	Not applicable
Frequency, duration and method of animal monitoring after dosing	Animals were monitored daily for mortality and clinical signs
Time and intervals of body weight determination	Fortnightly (weeks 2, 4 and 8)
Incubation, storing and hatching	Eggs removed daily, and incubated until day 21 then they were removed to a hatching tray until day 24.
Test period after egg-laying	14 days
Turning of eggs	Once daily
Collection of period for eggs	Daily

Table A7.5.3.1.3-4 Test Conditions (housing)

Criteria	Details
Test temperature	Average 25°°C
Shielding of the animals	Indoors
Ventilation	Not recorded
Relative humidity	Average 56%
Photoperiod and lighting	Sylvania Design 50 daylite spectrum lighting 17 hours per day
Storing, incubation and hatching conditions for eggs	Temperature 16 – 19°C. Relative humidity 58 – 67%
Environmental conditions for young birds	As for the adults

Table A7.5.3.1.3-5 Results

Parameter	Control	100mg/kg diet	500mg/kg diet	1000mg/kg diet
Mortality (male/female)	0 : 3	0 : 2	1 : 5	1 : 2
Food consumption (g/bird/day)	w 1 – 8: 13 – 14 w 21: 24	w 1 – 8: 14 – 17 w 21: 25	w 1 – 8: 13 – 15 w 21: 28	w 1 – 8: 13 – 14 w 21: 26
Bodyweight males (g/bird)	w 1: 225 w 21: 222	w 1: 211 w 21: 219	w 1: 205 w 21: 212	w 1: 207 w 21: 208
Bodyweight females (g/bird)	w 1: 211 w 21: 227	w 1: 214 w 21: 233	w 1: 210 w 21: 234	w 1: 208 w 21: 231
Number of eggs per hen	25	20	26	23
Egg shell thickness (mm)	0.233 ± 0.019	0.231 ± 0.015	0.231 ± 0.012	0.229 ± 0.017
Egg fertility (% viable embryos/ eggs set)	84.1	81.9	87.5	85.8
Normal hatchlings (% of eggs set)	62.9	62.1	70.6	66.4
14-d survivors (% of eggs set)	47.8	52.1	54.2	54.4
Reproductive success (no of 14-d survivors per hen)	10	8	11	10

Table A7.5.3.1.3-6 Validity Criteria for Bird Reproduction Test According to OECD 206

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	✓	
Average number of 14-day-old survivors per hen in controls ≥ 14, 12, 21 and 24 for mallard duck, bobwhite quail and Japanese quail		✓
Average eggshell thickness for the control group ≥ 0.34, 0.19 and 0.19 mm for mallard duck, bobwhite quail and Japanese quail	✓	
Concentration of the test substance in the diet ≥ 80% of the nominal concentration throughout the test period		✓

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	4 May 2007
Materials and methods	3.3.4 Test conditions Fo Generation Period (18 August 1991-7 November 1991). F1 Generation Growth Period (27 September 1991- 20 December 1991). On the 28 of august 1991, the first of an eventual 58 birds developed a region of excoration in either the tarsal/metatarsal areas, legs or anterior area of the head. Beginning on the 24 of October 1991, the birds with excoration were treated with a triple antibodies ointement (bacitracin-Neomycin-Polymyxin B) every day during the week and the severe cases were treated on weekends and holidays. Excoration was attributed to the increased activity and additional stress placed on the birds during the 17 hour lighting regime to promote egg production 4.2.1 Applied concentrations *Analysis showed that measured dietary concentration of fipronil in avian diet used in this study was between 41 and 118% of nominal*. On week two and twelve the measured concentration for the 0.2 mg./kg treatment is respectively 41.8 and 50.4 % of the nominal concentration. For the rest of the treatments and during the whole contamination period, measured concentration are >= to 80% of the nominal concentrations. 4.3 Results of controls The mean number of 14-day old survivors in the F1 generation per hen is 10 which is below the recommended value of 12 for the bobwhite quail, which are between 14 to 25 as the validity criterion is species specific. 5.3.2 Deficiencies see the above comments
Conclusions	Agree with the applicant
Reliability	2 As some of the measured concentrations and the number of 14-Day old survivors in the F1 generation per hen are below the recommanded values, the test is considered acceptable with restrictions
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.4 **Annex Point IIIA, XIII.3.1**	**Effects on honeybees**

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Acute toxicity to honeybees and other beneficial arthropods**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A.7.5.4.1/01** XXXX The acute oral and contact toxicity of M&B 46030 to honey bees. (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA l, 141-1 MAFF UK 1986	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil technical	
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	
3.1.3	Purity	95.4% w/w	
3.1.4	Composition of product	Not applicable	
3.1.5	Further relevant properties	None	
3.1.6	Method of analysis	Not applicable	
3.2	**Administration of the test substance**	**Contact (cuticular absorption)** One cage of bees at a time were lightly anaesthetised with carbon dioxide and a 1.0µl droplet of the appropriate dilution of test material was placed on the ventral surface of the thorax of each bee using a micrometer syringe. The bees were then replaced in the cage. Control groups were treated with a 1.0µl droplet of DMSO only.	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Acute toxicity to honeybees and other beneficial arthropods**

		Oral (normal ingestion) The appropriate concentration was administered as a single dose of 0.2ml to each group of 10 bees in a cage. The dose was introduced with a syringe into a glass tube 50 x 8 mm with a 1.5mm opening. The tube was inserted open end down through the top of the cage. The bees have been shown in laboratory studies to share the 0.2ml among themselves so that each received a similar dose of approximately 20 µl. A solution of the appropriate concentration of the test material in DMSO (1part) was mixed with 20% sucrose in water (19 parts). When the bee had taken all the test solution after approximately 4 hours the dosage tubes were replaced by tubes containing 20% sucrose.	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Test organisms	See Table A7.5.4-2	
3.4.2	Test system	See Table A7.5.4-3	
3.4.3	Diet	20% sucrose in water *ad libitum* (except during dosing in the oral test).	
3.4.4	Test conditions	See Table A7.5.4-4	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Mortality	
3.4.7	Examination / Observation	24 and 48 hours	
3.4.8	Statistics	The acute oral and contact LD50 values were calculated by probit analysis.	
		4. RESULTS	
4.1	**Limit test / Range finding test**	No	
4.2	**Results test substance**	See Table A7.5.4-5	
4.3	**Results of controls**	See Table A7.5.4-5	
4.4	**Test with reference substance**	No	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Acute toxicity to honeybees and other beneficial arthropods**

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: fipronil batch no PGS 963, purity 95.4% Test organisms: worker bees of *Apis mellifera*, 10 bees per cage, 2 cages per test item group and in the solvent control Temperature: 24 ±1°C Relative humidity: 50 ± 3% Lighting: conducted in the dark except fir essential procedures, Bees were fed 20% sucrose in water ad libitum (except during dosing in the oral test. Bees were exposed via an oral dose or by contact. Exposure levels: 0 (solvent control, DMSO), 0.002, 0.003, 0.0045, 0.0067 and 0.01µg/bee. Mortalities were observed and reported 24 and 48 hours after dosing. The acute oral and contact LD50 values were calculated by probit analysis.	
5.2	**Results and discussion**	The acute oral and contact LD50 values (and corresponding 95% confidence limits) were 0.00417 (0.0032 – 0.00494) and 0.00593 (0.00465 – 0.00756) µg/bee respectively	
5.2.1	LD_{50}	Oral 0.00417 µg/bee Contact 0.00593 µg/bee	
5.3	**Conclusion**		
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	

Table A7.5.4-1 Method of Administration of the Test Substance

Criteria	**Details**
Water	
Organic carrier	Dimethylsulphoxide (DMSO)
Concentration of the carrier [% v/v]	Calculated to give the appropriate concentration in a 1µl dose per bee (contact administration). Calculated to give the appropriate concentration in a 0.2ml dose per group of 10 bees (Oral administration) after dilution 1: 19 with sucrose solution
Other vehicle	20% sucrose in water solution (oral administration only)
Function of the carrier / vehicle	DMSO – Solvent sucrose solution – bait

Table A7.5.4-2 Test Animals

Criteria	Details
Species/strain	Honey bee (*Apis mellifera*) – sterile females (workers)
Source	XXXX
Age (in weeks), sex and initial body weight (bw)	Not determined
Breeding population	Not determined
Amount of food	20% sucrose solution (except during dosing)
Age at time of first dosing	Within 3 – 4 hours of removal from the hive
Health condition / medication	Not recorded

Table A7.5.4-3 Test System

Criteria	Details
Test location	Indoors
Holding pens	Cylindrical wire mesh cages 11.5 cm long x 4.0 cm in diameter
Number of animals	10 bees per cage
Number of animals per pen [cm^2/bee]	1.25
Number of animals per dose	20 (2 cages of 10 bees)
Pre-treatment / acclimation	None – bees were not fed before treatment
Diet during test	20% sucrose solution (except during dosing)
Dosage levels (of test substance)	Bees were exposed via an oral dose or by contact. Exposure levels: 0 (solvent control, DMSO), 0.002, 0.003, 0.0045, 0.0067 and 0.01µg/bee.
Replicate / dosage level	2
Feed dosing method	Contact : by direct application to the abdomen Oral : ingestion of a sucrose solution containing the treatment dose
Dosing volume per application	Contact : 1µl per bee Oral : 0.2 ml per 10 bee
Frequency, duration and method of animal monitoring after dosing	24 and 48 hours after dosing

Table A7.5.4-4 Test Conditions (housing)

Criteria	Details
Test temperature	24°C ± 1°C
Shielding of the animals	Study conducted indoors
Relative humidity	50 ± 3%
Photoperiod and lighting	Test were conducted in the dark except for essential procedures

Table A7.5.4-5 Results Data

Dose µg/bee	Mean mortality (%)			
	Oral		Contact	
	24 hours	48 hours	24 hours	48 hours
0 (solvent control)	0	5	0	5
0.002	0	10	0	5
0.003	10	35	5	20
0.0045	35	60	25	40
0.0067	70	80	30	60
0.01	90	100	45	75

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 24, 2006-
Materials and methods	Agree with the applicant
Conclusion	Agree with the applicant
Reliability	1
Acceptability	As the results are expresses in µg/bee, caution should be made when using them in the risk assessment.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Toxicity to non-target soil-dwelling arthropods**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.5.4.1/02** XXXX An Aged Residue Field Trial on the Effects of BAS 350 00 I on the Reproduction of Rove Beetles *Aleochara bilineata,* (unpublished) (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Grimm *et al.*, 2000: A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory conditions.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes. Test conditions: Test conditions were not electronically recorded during 5 days in 2 bioassays Temperature was slightly higher (+ 1.46 °C) for a short time (9.75 h) in the 7th bioassay.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Formulated product (Regent 800 WG) with a nominal content of 80% Fipronil	X
3.1.1	Lot/Batch number	130405	
3.1.2	Purity	80.3 % w/w	
3.1.3	Further relevant properties	None.	
3.1.4	Method of analysis	Associated soil residue study available (XXXX). Soil samples were analyzed according to BASF residue method 547/0.	
3.3	**Reference substance**	Perfekthion EC (Dimethoate 400 g/L nominal)	
3.3.1	Method of analysis for reference substance	Not applicable.	
3.4	**Testing procedure**		

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Toxicity to non-target soil-dwelling arthropods**	
3.4.1 Test organisms	See Table A7.5.4-2	X
3.4.2 Test design	An aged residue study was carried out to determine the duration of effects of Fipronil on the Staphylinid beetle *Aleochara bilineata.* The application of the test item and the aging of soil residues was carried out under field conditions, while the exposure was conducted under controlled laboratory conditions according to the mentioned guideline.	
	The test substance was applied on an arable field in-furrow (soil incorporated in a narrow band over and around the seeding row) at treatment rates of 50 g a.s./ha, 100 g a.s./ha and 200 g a.s./ha in water corresponding to nominal soil concentrations of 0.625, 1.25 and 2.5 mg/kg soil, respectively. Directly after treatment and after certain time intervals, soil samples were taken from the furrows (the treated area of the field) to expose the test species *A. bilineata* to fresh applied soil and to total soil residues aged for different time periods. In total, 7 samplings were conducted: One directly after treatment and further after 4, 10, 15, 20, 25 and 30 **W**eeks **A**fter **A**pplication. The soil samples were taken from the upper 10 cm soil layer using a soil sampler with a diameter of 4 cm in the furrows.	X
	Under laboratory conditions 7 bioassays were started exposing *A. bilineata* to the different soil samples. In addition to the test item a control and a toxic reference were set up. For the 1st bioassay also samples from between furrows (areas of the field not treated) were taken. In each bioassay, four replicates with 10 pairs of adult beetles (10 females and 10 males) were exposed to the soil for 28 days. After 7, 14 and 21 days after start of the respective bioassay approximately 500 pupae of *Delia antiqua* were distributed in the soil of each replicate to be parasitized of the F1-larvae of *A. bilineata*. After 28 days the adult beetles were separated from the soil and after 35 days the fly pupae were separated from the soil. The fly pupae were observed for hatching of the adult F1-generation. Thus, this study covers the whole life cycle of *A. bilineata*: parental generation, mating and oviposition of the parental generation, hatching of the F1-larvae, parasitization and hatching of the F1-adults. All stages of the life cycle were exposed to the treated soil. The reproduction of the beetles in the test item treatment was compared to that in the control.	
	In parallel an analytical study was carried out to determine the total residues of Fipronil in field soil samples (XXXX and XXXX).	
3.4.3 Diet	Frozen midge larvae	
3.4.4 Test conditions	See Table A7.5.4-4	X
3.4.5 Duration of the test	28 days of exposure in each bioassay, the last bioassay started 30 weeks after treatment.	X
3.4.6 Endpoint	Reproduction of *Aleochara bilineata*	X
3.4.7 Validity criteria	Mean number of emerged beetles in the control: > 400 beetles / replicate Reduction of reproduction in the toxic standard when compared to the control: at least 50 %.	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**		**Toxicity to non-target soil-dwelling arthropods**	
3.4.8	Statistics	Student t-test ($\alpha = 0.05$)	
		4. RESULTS	
4.1	**Limit test / Range finding test**	No.	
4.2	**Results test substance**	The results of each bioassay exposing A. bilienata to fipronil-treated soil clearly show that the effects on reproduction are dose related. It could clearly be demonstrated that the effects on reproduction are time depended with increasing reproduction through time. For the 50 g a.s./ha treatment, adverse effects on *A. bilineata* declined to acceptable levels after 10 weeks, corresponding to 0.479 mg/kg soil total residues. For the 100 g a.s./ha treatment, effects for *A. bilineata* declined to acceptable levels after 30 weeks, corresponding to 0.243 mg/kg total residues. Taken into account all available data acceptable effects were observed when *A. bilineata* was exposed to 0.243 mg total residues per kg soil. In all treatments no effects were observed when *A. bilineata* were exposed to soil samples taken between the furrows (soil non treated with fipronil). The results are summarized in Table A7.5.4-5.	X
4.3	**Results of controls**	Mean number of emerged beetle in the control: 1st bioassay (DAT 0): 779 beetles 2st bioassay (WAT 4): 611 beetles 3st bioassay (WAT 10): 677 beetles 4th bioassay (WAT 15): 713 beetles 5th bioassay (WAT 20): 788 beetles 6th bioassay (WAT 25): 708 beetles 7th bioassay (WAT 30): 780 beetles The validity criterion was met in all bioassays.	
4.4	**Test with reference substance**	Effect on reproduction compared to the control was 92.7% in the first bioassay at DAT 0. The validity criterion was met.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Test item: Fipronil (Regent 800 WG) batch no 130405, Test organisms: all life stages of the rove beetle *A. bilineata.* The test substance was applied on an arable field at treatment rates of 50 g a.s./ha, 100 g a.s./ha and 200 g a.s./ha in water corresponding to soil concentrations of 0.625, 1.25 and 2.5 mg/kg soil, respectively. Directly after treatment and after certain time intervals, soil samples were taken from the furrows to expose the test species *A. bilineata* to fresh applied soil and to total soil residues aged for different time periods. In total, 7 samplings were conducted: One directly after treatment and further after 4, 10, 15, 20, 25 and 30 Weeks After Application. The soil samples were taken from the upper 10 cm soil layer using a soil sampler with a diameter of 4 cm in the furrows.	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**		**Toxicity to non-target soil-dwelling arthropods**	
		Under extended laboratory conditions 7 bioassays were started exposing *A. bilineata* to the different soil samples. In addition to the test item a control and a toxic reference were set up. For the 1st bioassay also samples from between furrows were taken. In each bioassay, four replicates with 10 pairs of adult beetles (10 females and 10 males) were exposed to the soil for 28 days. After 7, 14 and 21 days after start of the respective bioassay approximately 500 pupae of *Delia antiqua* were distributed in the soil of each replicate to be parasitized of the F1-larvae of *A. bilineata*. After 28 days the adult beetles were separated from the soil and after 35 days the fly pupae were separated from the soil. The fly pupae were observed for hatching of the adult F1-generation. Thus, this study covers the whole life cycle of *A. bilineata*: parental generation, mating and oviposition of the parental generation, hatching of the F1-larvae, parasitization and hatching of the F1-adults. All stages of the life cycle were exposed to the treated soil. The reproduction of the beetles in the test item treatment was compared to that in the control. In parallel an analytical study was set up to determine the total residues of Fipronil in field soil samples (XXXX and XXXX).	
5.2	**Results and discussion**	The results of each bioassay clearly show that the effects on reproduction are dose related. It could clearly be demonstrated that the effects on reproduction are time depended with increasing reproduction through time. For the 50 g a.s./ha treatment, effects on *A. bilineata* declined to acceptable levels after 4 weeks, corresponding to 0.479 mg/kg soil total residues. For the 100 g a.s./ha treatment, effects on *A. bilineata* declined to acceptable levels after 20 weeks, corresponding to 0.243 mg/kg total residues. Taken into account all available data acceptable effects were observed when *A. bilineata* was exposed to 0.243 mg total residues per kg soil. In all treatments no effects were observed when *A. bilineata* were exposed to soil samples taken between the furrows (soil non treated with fipronil). The results are summarized in Table A7.5.4-5.	X
5.2.1	NOEC	0.243 mg / kg soil	X
5.3	**Conclusion**	The effects on reproduction clearly demonstrate a dose response relationship. No effects at all were observed when *A. bilineata* was exposed to soil samples taken between furrows in all treatments. No unacceptable adverse effects were observed, when *A. bilineata* was exposed to soil samples with a concentration of 0.243 mg/kg total residues of Fipronil.	X
5.3.1	Reliability	1	X
5.3.2	Deficiencies	None	X

Table A7.5.4-1 Application of the Test Substance

Criteria	**Details**
Application rates	50, 100 and 200 g a.i./ha corresponding to 0.625, 1.25 and 2.5 mg a.i./kg soil in 175 L/ha water.
Application technique	Plot application system (type PSG-System 2, Schachtner Gerätetechnik, D-71640 LU, Germany). The sprayer was equipped with an extension tube including 4 spraying nozzles (Teejet 4002 E) with a distance of 75 cm between each nozzles. The test item was directly applied into furrows of 4 cm width.
Application of the test rates	The test item was sprayed into furrows with a width of 4 cm on arable land. The soil assigned to the control group was left untreated, but under the same conditions as the test item treated soil. The reference item was applied at a rate of 4.4 L/ha on untreated soil from the test site.

Table A7.5.4-2 Test organisms

Criteria	**Details**
Species/strain	Rove beetle (*Aleochara bilineata* Gyll., Staphylinidae)
Source	XXXX
Age (in days), sex	Adults, 1 to 6 days old, females and males
Food and amount	Frozen midge larvae, 3 x per week *ad libitum*
Host organism for parasitization	*Delia antiqua* pupae (Diptera, Anthomyiidae)
Source	De groene Vlieg, NL

Table A7.5.4-3 Test System

Criteria	Details
Test location	Laboratory: IBACON, Roßdorf, Germany
Test units	Plastic boxes filled with natural soil and covered with perforated plastic lids were used for exposure. After 35 days of exposure the fly pupae parasitized by the F1 larvae were placed in a funnel, which was placed on a glass beaker. The bottom of the funnel was perforated with holes through which the emerged beetles fall into the glass cylinder. The pupae remained in the funnel.
Test substrate	850 g natural soil per test unit. During exposure the soil was moistened to about 35 +/- 5 % maximum water holding capacity.
Number of animals per replicate	10 pairs (10 females + 10 males)
Number of replicate per treatment	4
Acclimatisation	Beetles arrived as parasitized fly pupae. They were transferred to a funnel, which was placed on a glass beaker. The bottom of the funnel was perforated with holes to ensure that emerged beetles fell into the glass beaker and the pupae remained in the funnel. The emerged beetle were counted and separated daily to ensure the age of the test beetles. After that the beetles were held in plastic boxes on moistened tissue paper under test conditions.
Diet during test	Frozen midge larvae; 3 x per week *ad libitum*
Test item rate	0.625, 1.25 and 2.5 mg a.s./kg soil

Table A7.5.4-4 Test Conditions

Criteria	Details
Test temperature in the laboratory during bioassay	18°C – 23°C
Relative humidity during bioassay	60 – 90 %
Light intensity during bioassay / photoperiod	379 – 1360 lux; 16 h light: 8 h dark
Climatic conditions during ageing of the soil residues	Natural climatic conditions

Table A7.5.4-5 Effects of total[3)] soil residues of Fipronil to the rove beetle *Aleochara bilineata* under extended laboratory conditions

Test species Substrate	Samplings [1)] [DAT / WAT]	Nominal Concentration in soil [mg a.s./kg] [2)]	Measured Concentration in soil [mg a.s./kg] [3)]	Effects sublethal [%] [4)]	References
Aleochara bilineata Natural soil, field furrow application – aged residue	EXPOSURE TO SOIL IN-FURROW				
	DAT 0	0.625	0.489	58.3	Bioassays: 2005/1020069 Analytics: 2005/1018553 and 2005/1022458
	WAT 4		0.479	41.1	
	WAT 10		0.241	33.8	
	WAT 15		0.174	22.7	
	DAT 0	1.250	1.007	97.0	
	WAT 4		1.042	98.8	
	WAT 10		1.156	80.9	
	WAT 15		0.464	67.3	
	WAT 20		0.243	34.1 [5)]	
	WAT 25		0.471	39.5	
	WAT 30		0.486	23.1	
	DAT 0	2.500	2.081	99.9	
	WAT 4		1.733	100.0	
	WAT 10		2.916	100.0	
	WAT 15		1.083	98.9	
	WAT 20		0.922	69.1	
	WAT 25		0.992	91.3	
	WAT 30		1.106	70.3	
Aleochara bilineata 4) Natural soil, field furrow application – aged residue	EXPOSURE TO SOIL BETWEEN-FURROW				
	DAT 0	0.625	--	3.2	
	DAT 0	1.250	--	-3.6	
	DAT 0	2.500	--	1.9	

DAT = **D**ays **A**fter **T**reatment; WAT = **W**eeks **A**fter **T**reatment
The calculation is based on a furrow width of 4 cm, a soil depth of 10 cm and a soil density of 1.5 g/cm³ (dry weight).

Mean measured concentrations based on the analytical determination of total (fipronil + XXXX + XXXX + XXXX) soil residues in the field (XXXX). To be able to compare the measured residues in soil with the concentrations to which *A. bilineata* was exposed, a correction has to be made for the soil concentrations measured in the analytical report. For the soil samples used in the analytical measurements, a soil sampler of 5 cm diameter was used, and in the biological studies, soil samplers of 4 cm diameter were used. Since the width of the treated furrow is 4 cm, the sampler in the analytical study exceeds that of the applied furrow. Sampling in the analytical study obviously leads to soil concentrations lower than the actual exposure concentrations in the biological study. Therefore, the measured values in XXXX were multiplied by a factor of 1.25 (estimated from the ratio 5:4 cm of sampler width and furrow width). To define the worst-case exposures (*i.e.* the lowest concentrations of total soil residues, where effects on the tested species become to acceptable levels) for *A. bilineata*, the level of acceptable effects was compared to the pertaining total soil residues (*i.e.* the sum of total residues of fipronil + metabolites as measured in study XXXX (and XXXX), multiplied with 1.25). The metabolite XXXX was never detectable above the quantification limit of 0.2 µg/kg and was therefore not included.

The test system is not practicable for assessing mortality (*i.e.* lethal effects), because the beetles must not be disturbed during the test. Therefore, the test system focussed on reproduction, which is the relevant endpoint that has to be considered in *A. bilineata* studies. According to Grimm et al. (2000) it is indeed recommended to record the number of dead or alive beetles, but these data are not necessary to evaluate the test.

No unacceptable effects according to ESCORT II (European Standard Characteristics of Non-Target Arthropod Regulatory testing) criteria.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 21, 2006
Materials and methods	The applicant's version is adopted with the following revisions: 3.4.2 Test Design One day before application, the field arable was tilled with a rotary tiller and the soil tunred using a cultivator. The maize was sown with a maize drill embedding the grains in the furrow into a depth of 5 cm. The sowing rate was ca 85,000 to 90,000 seeds/ha. The test item was applied during maize in the furrows **(*soil incorporated in a narrow band over and around the seeding row) at treatment rates of 50 g a.s./ha, 100 g a.s./ha and 200 g a.s./ha (with four replicates per application) in water corresponding to nominal soil concentrations of 0.625, 1.25 and 2.5 mg/kg soil, respectively.*** The application was carried out directly after depositing the seed in the soil in the opened furrow. After application, the furrow was completely covered with soil by the sowing machine. ***Directly after treatment and after certain time intervals, soil samples were taken from the furrows (the treated area of the field) to expose the test species A. bilineata to fresh applied soil and to total soil residues aged for different time periods. In total, 7 samplings were conducted: One directly after treatment and further after 4, 10, 15, 20, 25 and 30 Weeks After Application. The soil samples were taken from the upper 10 cm soil layer using a soil sampler with a diameter of 4 cm in the furrows.*** Before testing began, the soil samples were deep frozen for at least 12 hours in order to defaunate them. 3.4.4 Test Conditions To be added in Table A7.5.4-1 Application of the Test Substance Plot size : for each application 4 experimental plots of 108 m^2 were treated Soil profile of the field : middle silt sand with a WHC = 45 % 3.4.5 Duration of the tests ***28 days of exposure in each bioassay, the last bioassay started 30 weeks after treatment*** Change to***:*** The adult test organisms were exposed to the test item for 28 days. After 28 days, all surviving adult beetles were removed from the soil and the nulber recorded. The soil and the parasitized onion fly pupae were returned to the climatic room in the original test units for a further week. 35 days after application the pupae were washed out of the soil and the pupae of each replicate were transferred into two separate emergence container. Emergence of the beetles was then monitored. The test were considered finished after the control treatment fell below a rate of two emerged beetles per replicate per day. 3.4.6 Endpoint ***Reproduction of Aleochara bilineata***. To be added : the reproductive capacity was assessed as the number of beetles of the F1-generation hatched form the offered pupae. For the determination of the effect on reproduction , the mean number of offspring of each treatment group was calculated by averaging the

	number of offspring in each replicate of that treatment group. The effect on reproductive performance relative to the control was derived using the following formula: R = (1-T/C)* 100 T: absolute number of emerged beetles in the test item C: absolute number of emerged beetles in the control
Results and discussion	The applicant's version is adopted with the following revisions: 5.2 Results and discussion ***The results of each bioassay clearly show that the effects on reproduction are dose related. It could clearly be demonstrated that the effects on reproduction are time depen***dant ~~***ded***~~ also as a decrease of the effects on reproduction is observed though time ~~***increasing reproduction through time.***~~ ***For the 50 g a.s./ha treatment,*** a statistically significant decrease on reproductive performance of A. bilineata compared to the control is observed until WAT 15 **(**WAT =**W**eeks **A**fter **T**reatment) ~~***Declined to acceptable levels after 4 weeks, corresponding to 0.479 mg/kg soil total residues.***~~ ***For the 100 g a.s./h*** and the 200 g a.s./h ***treatments,*** a statistically significant decrease on reproductive performance of A. bilineata compared to the control is observed until WAT 30 **(**WAT =**W**eeks **A**fter **T**reatment) ~~***effects on A. bilineata declined to acceptable levels after 20 weeks, corresponding to 0.243 mg/kg total residues***~~ ~~***Taken into account all available data acceptable effects were observed when A. bilineata was exposed to 0.243 mg total residues per kg soil. In all treatments no effects were observed when A. bilineata were exposed to soil samples taken between the furrows (soil non treated with fipronil).***~~ According to the Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2 workshop, March 2000), a 30% threshold value for acceptable effects was chosen. For the 50 g a.s./ha treatment, the threshold value was obtained after 10 Weeks (WAT 10), corresponding to a mean measured concentration of 0.241 mg/kg of soil residue For the 100 g a.s./ha treatment, the threshold value was obtained after 20 Weeks (WAT 20), corresponding to a mean measured concentration of 0. 243 mg/kg of soil residue For the 200 g a.s./ha treatment, the threshold not obtained during the test. 5.2.1.~~***NOEC***~~ Threshold value The 30% threshold value was obtained when the concentration of fipronil was 0.243 mg / kg soil.
Conclusion	
Reliability	2
Acceptability	The test is considered acceptable.
Remarks	

COMMENTS FROM …
Date
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Toxicity to non-target soil-dwelling arthropods**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A7.5.4.1/03** **NON KEY STUDY** XXXX: Effects of EXP60720A on the reproduction of Rove Beetles *Aleochara bilineata* Gyll. (Coleoptera, Staphylinidae) in the Laboratory. (XXXX)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF	
1.2.2	Companies with letter of access	None	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes - IOBC approved method of Moreth & Naton (1992); - improvements of the *Aleochara* ring-test group (Grimm *et al.* 2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	X
		3. MATERIALS AND METHODS	
3.1	**Test material**	EXP60720A, WG-type formulation containing fipronil at 800 +/- 25 g/kg	
3.1.1	Lot/Batch number	Lot No.: OP200908	
3.1.2	Purity	798 g a.s./kg analysed	
3.1.3	Further relevant properties	Dispersible in water	
3.1.4	Method of analysis	Not reported	
3.3	**Reference substance**	Perfekthion EC (BAS 152 11 I), containing 400 g/L Dimethoate	
3.3.1	Method of analysis for reference substance	Not reported	
3.4	**Testing procedure**		
3.4.1	Test organisms	Adult *Aleochara bilineata*, 1-4 days old at test initiation (see Table A7.5.4-2)	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Toxicity to non-target soil-dwelling arthropods**	
3.4.2 Test design	Exposure of the adult beetles was reached via treated quartz sand. 10 males and 10 females per replicate were exposed to 5 concentrations of the test item, to a deionised water control and to a toxic standard. 4 replicates per treatment group were set up (see Table A7.5.4-3). After 7, 14 and 21 days after treatment the newly hatched F1-larvae were supplied with the host organism *Delia antiqua* (onion fly) for parasitation. Accordingly, 750 pupae of the host were added to the soil per replicate and day. 35 days after application the pupae were sieved out of the sand for emergence of the beetles.	X
3.4.3 Diet	Frozen midge larvae	
3.4.4 Test conditions	See Table A7.5.4-4	
3.4.5 Duration of the test	97 days	
3.4.6 Endpoint	Reproduction of *Aleochara bilineata*, calculation of an ER_{50}	
3.4.7 Validity criteria	Mean number of emerged beetles in the control: > 400 beetles / replicate Reduction of reproduction in the toxic standard when compared to the control: at least 50 %.	
3.4.8 Statistics		
	4. RESULTS	
4.1 Limit test / Range finding test	No	
4.2 Results test substance	Behavioural abnormalities: no test item related behavioural abnormalities were observed. Emergence of young *A. bilineata* per container: at concentrations up to 10.0 g a.i./ha, reduction in reproduction was not statistically significant compared to the control (see Table A7.5.4-5). At concentrations ≥ 31.6 g a.i./ha, emergence was statistically significantly reduced. The ER_{50} was calculated to be ER_{50} = 35.93 g a.i./ha;	
4.3 Results of controls	740 – 1012 beetles emerged in the 4 replicates (mean: 917, *i.e.* 40.7 % of the 9000 introduced pupae in the control group were parasitized). The validity criterion was met.	
4.4 Test with reference substance	Effect on reproduction compared to the control was 81.5 %. The validity criterion was met.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Test item: EXP60720A, WG-type formulation (lot OP200908) containing fipronil at 800 +/- 25 g/kg (certified content 798 g/kg) Test organisms: all life stages of the rove beetle *A. bilineata.*	

Section A7.5.4.1 **Annex Point IIIA, XIII.3.1**	**Toxicity to non-target soil-dwelling arthropods**	
	The test organisms (10 males and 10 females each per unit, 4 replicates per treatment group) were exposed on quartz sand to concentrations of 0 (control), 1.0, 3.16, 10.0, 31.6, 100.0 g a.i./ha and to a toxic standard for 28 days under worst-case laboratory conditions. On day 7, 14 and 21 after test initiation, approximately 750 fly pupae (*Delia antiqua*) were added to each replicate as hosts for parasitization by the juveniles produced during the test. Frozen midge larvae were provided as food ad libitum during the test. After 28 days, the adult test organisms were removed. After 35 days, all fly pupae were sieved from the sand and transferred into separate emergence containers. After 97 days, the reproductive performance of the exposed beetles per replicate was assessed as the total number of juvenile beetles hatching from the parasitized fly pupae. The ER_{50} (median effect rate) based on the reproductive performance and its confidence limits were estimated by applying Probit Analysis. The statistical significance of differences in the reproductive performance between the test substance treated groups and the controls was evaluated with the Dunnett's-test (α = 0.05).	
5.2 Results and discussion	The ER_{50} was calculated to be 35.93 g a.i./ha (95% confidence limits 23.8 - 59.6 g a.i./ha), equivalent to 0.078 mg a.i./kg sand. Statistically significant effects on the reproductive performance were observed at 31.6 g a.i./ha and above. No significant effects were observed at 1.0, 3.16 and 10.0 g a.i./ha. The results are summarized in Table A7.5.4-5.	
5.2.1 ER_{50}	35.93 g a.i./ha, corresponding to 0.078 mg a.i./kg sand	
5.3 Conclusion		
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A7.5.4-1 Application of the Test Substance

Criteria	Details
Application rates	1.0, 3.16, 10, 31.6 and 100 g a.i./ha (equivalent to 2.18, 6.88, 21.8, 68.8 and 217.6 µg a.i./kg sand). Concentration of the test item per unit: 2.32, 7.31, 23.13, 73.13, 231.3 µg product/test unit.
Application technique	Spray application
Application of the test rates	The test item was dispersed in the water necessary for adjusting the water amount of the quartz sand (*i.e.* 10% v/v) and was evenly mixed into the sand with a laboratory mixer.

Table A7.5.4-2 Test organisms

Criteria	**Details**
Species/strain	Rove beetle (*Aleochara bilineata* Gyll., Staphylinidae)
Source	XXXX
Age (in days), sex	Age at test start: 1 – 4 days old adults, males / females
Food and amount	Frozen midge larvae (commercial food for aquarium fish; *Chironomus spec.*, Zoohaus Dieburg, Frankfurter Str. 69, Dieburg, Germany), every working day *ad libitum*
Host organism for parasitization	*Delia antiqua* Meig. pupae (Dipera, Anthomyiidae)
Source	XXXX

Table A7.5.4-3 Test System

Criteria	**Details**
Test location	XXXX
Test units	Exposure units: plastic boxes (18.3 cm x 13.6 cm x 6 cm) covered with perforated plastic lids Emergence containers: funnel (height: 8 cm, diameter: 13 cm), which was placed on a glass beaker (height: 14 cm, diameter: 8 cm); the bottom of the funnel was perforated with holes (diameter approximately 2 mm) through which the hatched beetles fell into the glass beaker below. The pupae remained in the funnel.
Test substrate	Quartz sand: particle size 0.1 mm to 0.5 mm
Number of animals per replicate	10 males and 10 females
Number of replicate per treatment	4
Acclimatisation	The parasitized fly pupae were transferred in emergence containers (see above). The emerged beetles were counted and separated daily to ensure the age of the test beetles. After counting they were held in plastic boxes on moistened tissue paper under test conditions for 1 – 4 days before test initiation. Food was added *ad libitum*.
Diet during test	Frozen midge larvae were fed every working day *ad libitum*
Test item rate	1.0, 3.16, 10, 31.6 and 100 g a.i./ha (equivalent to 2.18, 6.88, 21.8, 68.8 and 217.6 µg a.i./kg sand)

Table A7.5.4-4 Test Conditions

Criteria	Details
Test temperature in the laboratory during bioassay	During exposure: 19 – 22 °C Post-exposure: 17 – 23 °C
Relative humidity during bioassay	During exposure: 40 – 90 % Post-exposure: 40 – 95 %
Light intensity during bioassay / photoperiod	During exposure: 520 – 870 lux Post-exposure: 520 – 1480 lux
Climatic conditions during ageing of the soil residues	-

Table A7.5.4-5 Effects of Fipronil to the rove beetle *Aleochara bilineata* in a worst-case laboratory trial

Test concentration [µg a.i./kg soil]	Control	2.18	6.88	21.8	68.8	217.6	Toxic standard
Emerged beetles per replicate (mean +/- SD)	917 ± 123	1042 ± 64	904 ± 112	744 ± 86	522 ± 94 *	184 ± 23 *	170 ± 24 *
Reduction compared to the controls [%]	n.a.	- 13.7	1.3	18.8	43.0	80.0	81.5

n.a. not applicable
* statistically significant reduction compared to the control; Dunnett-test, $\alpha = 0.05$

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Materials and methods	Agree with the applicant's version. **Revisions/amendments:** **2.3 Deviations :** some deviations to the study protocol were recorded : - temperature > 22°C and < 18°C during post exposure period, - relative humidity > 90% or <60% during exposure and post exposure periods; - no temperature and relative humidity recorded for 2 days during post application period. None effect of these deviations on the study was presumed. **3.4.2 Test design :** *Exposure of the adult beetles was reached via treated quartz sand. 10 males and 10 females per replicate were exposed to 5 concentrations of the test item, to a deionised water control and to a toxic standard.* The 10 pairs of beetles were introduced into the test units immediately after application. See also Table A7.5.4-1 for the application of the test substance. *4 replicates per treatment group were set up (see Table A7.5.4-3).* ~~After~~ *7, 14 and 21 days after treatment the newly hatched F1-larvae …*
Conclusion	Agree with the applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	As explained by the applicant in the document 'COMPLEMENTARY INFORMATION TO THE BIOCIDE SUBMISSION OF FIPRONIL (PT 18) – Non-Target Arthropods' presented below, this second endpoint on *A. bilineata* was measured in a worst-case laboratory study, exposing the test organisms on inert substrate (quartz sand) under controlled, worst-case laboratory conditions. The first study (XXXX) summarized under point 7.5.4.1/02, which is a higher tier study, was carried out under more realistic conditions with an application made in the field on natural soil. Since higher tier studies reflect more realistic exposure conditions, the endpoints of such studies are considered to be the basis for a risk assessment. For that reason, the result of the study XXXX will not be taken into account for the risk assessment.
	COMMENTS FROM …
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Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

FIPRONIL BIOCIDE DOSSIER

COMPLEMENTARY INFORMATION TO THE BIOCIDE SUBMISSION OF FIPRONIL (PT 18) – Non-Target Arthropods

BASF Aktiengesellschaft

31 July 2007

Author
Andrea Drexler
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1. Introduction

The purpose of this document is to address the demand by AFSSET in its evaluation report of the Fipronil Biocide Dossier (PT18) for complementary information concerning the submitted non-target arthropod studies. A justification for choosing study XXXX as key study should be given, although the study XXXX produced a lower endpoint.

2. Data requirements

According to the biocide Directive 98/8/EC, data on non-target arthropods should be submitted for PT18, if the product is used outside buildings (see chapter 2.5, point 7.5.3.2 and more detailed in chapter 3, point 7.5.4.1). For these outside uses, data on one beneficial arthropod other than honeybees has to be provided. For in-door uses, no data on non-target arthropods are required due to the lack of exposure.

The dossier submitted is for an **in-door use** of fipronil (Goliath Gel) against cockroaches. The application will be done with a gel applicator throughout the infested area in houses as single small drops. Thus, according to the requirements of the biocide Directive 98/8/EC and the lack of exposure to the environment, **submission of non-target arthropod data and risk assessment is not required**.

For completeness sake and to show the intrinsic toxicity, endpoints from worst-case laboratory studies for three non-target arthropod species were provided (see Doc II A Table 4.2/6; references XXXX, XXXX, XXXX). For the most sensitive species (*i.e. Aleochara bilineata*), an additional, higher tier study (XXXX) was submitted to show the acceptability of initial effects under more realistic conditions.

3. Relevant endpoints

Four studies including data on three relevant non-target arthropod species (soil-dwelling predators *A. bilineata*, *Poecilus cupreus* and *Pardosa sp*.) were provided within the dossier. For each species, a worst-case laboratory study (XXXX, XXXX, XXXX) is available (see Doc II Table 4.2./6 and IUCLID). Considering these data, *A. bilineata* was the most sensitive species. Therefore, a higher tier study was carried out with this species with exposure under more realistic conditions (XXXX), considering that tests carried out with the most sensitive species cover less sensitive organisms.
Hence, two endpoints for *A. bilineata* are available. The first of the two endpoints discussed here (XXXX) was measured in a worst-case laboratory study, exposing the test organisms on inert substrate (quartz sand) under controlled, worst-case laboratory conditions. The aim of the study is to produce an LR_{50} to show the intrinsic toxicity of the compound. The second study (XXXX) was carried out under more realistic conditions. In this study, the application was made in the field on natural soil. Since the study was designed to determine the time necessary for a potential re-colonisation/recovery of possibly affected populations, soil samples were taken at defined time points after the application and transferred to the laboratory, where test organisms were exposed. To study the degradation/dissipation of the compound, the soil samples were analyzed for fipronil and its metabolites simultaneously. So it was possible to determine the actual exposure of the test organisms to the sum of fipronil

and its metabolites (*i.e.* total residues), and thus, it was possible to determine a soil concentration, where no unacceptable effects are observed. This concentration was determined to be 0.243 mg total fipronil residues per kg soil.

Effects of insecticide on non-target arthropods are obvious under worst-case conditions. Therefore, higher tier tests are carried out to study the effects under more realistic conditions. Usually, this leads to lower toxicity of the active ingredient due to for example changes in the availability of the compound. Since these higher tier studies usually reflect more realistic exposure conditions, the endpoints of such studies are considered to be the basis for a risk assessment, if necessary.

4. Conclusion

Considering the data requirements of biocide Directive 98/8/EC, data submission and a risk assessment for non-target arthropods are not necessary for the intended use of Goliath Gel, since no exposure of non-target arthropods in the environment will occur. However, data on four non-target arthropod-studies were submitted to get an impression of the intrinsic toxicity of fipronil under worst-case conditions and the acceptability of effects under more realistic conditions.

In any case, for risk assessment purposes, the relevant endpoint is 0.243 mg total fipronil residues per kg soil from the higher tier study XXXX.

Section A7.5.5 **Annex Point IIA, VII.7.5**	**Bioconcentration terrestrial**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Please see the "General comment on studies summarized in this section" A7.5	
Undertaking of intended data submission [ ]		

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Section A7.5.6 **Annex Point IIIA, XIII.3**	**Effects on other terrestrial non-target organisms**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Please see the "General comment on studies summarized in this section" A7.5.	
Undertaking of intended data submission [ ]		

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Section A7.5.7 Effects on mammals **Annex Point IIIA, XIII.3.4**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [x]**	
Detailed justification:	Please see the "General comment on studies summarized in this section" A7.5	
Undertaking of intended data submission [ ]	–	

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Section 7.6 **Annex Point IIA, VII.7.8** **IIIA, XII.4-XIII.5**	**Summary of ecotoxicological effects and fate and behaviour in the environment**

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Fate and Behaviour in water

Hydrolysis
Fipronil has been shown to be hydrolytically stable at environmentally relevant pH values.

The hydrolytic stability of [^{14}C]-Fipronil, was studied in the dark, under sterile conditions, at pHs 5, 7 and 9. At pHs 5 and 7, TLC and HPLC data showed that fipronil was hydrolytically stable. At pH 9 the rate of conversion is best modelled by pseudo-first order kinetics with a half life of 28 days and a rate constant k = 0.0243 day^{-1}.

Photolysis
The photolysis in water of [^{14}C]-Fipronil, was studied at pH 5 at 25 ± 1°C, under sterile conditions. Two degradation products were formed: the major organic extract photo-product was XXXX (43.4 % of the applied radioactivity) and a minor component (HPLC RT = 2 min) accounting for 4.0% of applied radioactivity. The kinetics of photolytic degradation were first order with a half-life of 3.63 hours under the xenon lamp corresponding to 0.33 days of summer sunlight in Florida and a rate constant k = -0.0176 days^{-1}. Photolysis can be considered a major route of fipronil degradation should it reach the aqueous environment.

Ready Biodegradability
Fipronil attained 47% degradation after 28 days. According to OECD criteria a test material may be considered to be readily degradable if > 60% degradation is attained after 28 days. Therefore, since there was only 47% degradation, fipronil cannot be considered readily degradable under the strict terms and conditions of the OECD guidelines.

Fate and Behaviour in Soil

Adsorption/desorption
The soil adsorption/desorption properties of [^{14}C]-Fipronil were investigated using five European soil types using the slurry technique. The adsorption constants (K) obtained ranged from 4.19 in a UK sandy loam to 20.69 in a UK loam. The value of K increased with increasing organic carbon content of the soil suggesting that more fipronil was adsorbed. The K_{OC} values obtained ranged from 427 to 1248 with a mean of 727. The Freundlich desorption constants increased with the increasing desorption cycles, the results suggest that the adsorption was reversible with similar processes involved in the desorption as the adsorption. . The results indicated that fipronil is unlikely to demonstrate significant mobility in soil due to its relatively high sorption t soil. According to McCall's designation, fipronil would be expected to show medium to low mobility.

Water/sediment degradation
A [^{14}C]-Fipronil degradation in two water/sediment systems showed that in an aerobic aquatic environment, fipronil partitions steadily into the underlying sediment where it degrades by reduction to XXXX. XXXX is further degraded by hydrolysis to XXXX. Fipronil is also hydrolysed to XXXX and, to a much lesser extent oxidised too XXXX. There is evidence than XXXX and XXXX are further transformed to RPA 10530 via oxidation or hydrolysis respectively. The results of this study show that fipronil will not persist in an aerobic aquatic environment.

Aerobic degradation in soil
The degradation of [^{14}C]-Fipronil was investigated in two soils. The half life of [^{14}C]-Fipronil determined by HPLC in a UK sandy loam soil and a German sandy soil under aerobic conditions were 128 and 308 days respectively, degradation proceeded via hydrolysis to XXXX and oxidation to XXXX.

Aaerobic degradation further study
In a study of [^{14}C]-Fipronil degradation in four soils (at 20°C) and two soils (at 10°C)
Fipronil was steadily degraded under aerobic conditions by hydrolysis to XXXX and by oxidation to XXXX. The rate of degradation was temperature dependent with more rapid degradation at 20°C than 10°C. The rate of degradation was also related to the soil microbial biomass activity.

The reduced metabolite XXXX was found in minor quantities, except in one soil where there was reduced oxygen status under these laboratory conditions. Several other minor metabolites were also observed, the hydrolysis products RP 200761 and XXXX in the high pH and high biomass soil.

The DT_{50} of fipronil ranged from 26 to 296 days at 20°C and the DT_{90} from 85 to 982 days. It was not possible to derive the DT values for the metabolites.

Soil mobility
In a column leaching study on five European soils fipronil was shown to have a low mobility in soil.

Soil dissipation northern europe
The environmental behaviour of fipronil and its metabolites was studied following the soil incorporation of an experimental wettable powder at two location in Northern Europe.

The trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to less than half in mid application samples (6 to 9 months after application) and below or close to the limit of quantification (LOQ), when averaged over the soil profile to 30 cm, one year after application at the two sites.

The major metabolite found was XXXX with significant amounts of XXXX. XXXX residues generally peaked at the mid-way sampling point between applications but residues were still present one year after each application. The amounts of XXXX residue were less than for XXXX but the trend was similar. The pattern of residues for XXXX was similar to XXXX but the amounts of XXXX found were only about 10 to 20% of XXXX. Residues of the photolyte, XXXX, remained below LOQ for both trials, which is consistent with soil incorporation of the parent compound.

The residues of the metabolites XXXX, XXXX and XXXX which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.

Soil dissipation southern europe
The environmental behaviour of fipronil and its metabolites was studied following the soil incorporation of an experimental wettable powder at two location in Southern Europe.

Based on the analytical results available up to the fifth and sixth years for the trials in France and Italy respectively, the trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to below LOQ by one year after application at both trial sites. No residues of the photolyte, XXXX, were found (apart from 2 detects) which is consistent with soil incorporation of the parent compound. The residues of the metabolites XXXX, XXXX and XXXX which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.

Photo-oxidation
An estimation of the degradation of Fipronil by photo-oxidation in air demonstrated that although unlikely to reach the air due to a low vapour pressure value and due to application by soil incorporation or bait should fipronil reach the air, it will be rapidly degraded in the troposphere with a half-life of 0.111 day assuming 12 hours of sunlight.

ECOTOXICOLOGICAL EFFECTS

The database of ecotoxicological studies with aquatic and terrestrial species conducted with fipronil is extensive and includes many more studies than those that have been summarized in full in this dossier. Full summaries (section IIIA 7.4 and 7.5) have been carried out for representative species proposed by the Biocide Products Directive (BPD 98/8/EC) and/or for the most sensitive freshwater species within each group. The same approach of summarizing only the most sensitive species has also been followed for terrestrial organisms. The results of these studies provide key information for the characterization of the toxicity profile of fipronil to freshwater aquatic organisms and terrestrial species. A full summary of all studies available for fipronil is not deemed necessary because the proposed use and application method of the fipronil biocidal product Goliath Gel leads to negliglible exposure in natural environments and therefore a risk assessment is not warranted.

Effects on aquatic organisms

Acute toxicity to fish
The acute toxicity of fipronil to juvenile Bluegill sunfish (*Lepomis macrochirus*) was studied under laboratory conditions during 96 hours of flow-through exposure. The 96-h LC_{50} was calculated at 85.2 µg a.s./l. Based on the absence of lethal or sublethal effects observed up to this concentration, the 96-h NOEC was determined at 43.2 µg a.s./l.

Acute toxicity to invertebrates
The acute toxicity of fipronil to neonate *Daphnia magna* (<24 hours old) was studied in a 48 hour flow through test in the laboratory. Treatment related immobilisation and lethargy among surviving daphnids were observed at 160 and 280 µg a.s./l. The 48-h EC_{50} was calculated at 190 µg a.s./l and the NOEC was 52 µg a.s./l.

Daphnids are less sensitive to fipronil than other invertebrates, particularly insects. Therefore, testing with fipronil has been conducted on additional species of aquatic invertebrates, including insects. The acute toxicity of fipronil to the Mayfly *Hexagenia spp* was studied in a 96-h static renewal laboratory test. The 96-h LC_{50} value derived from this study was calculated to be 0.44 µg a.s./l and the NOEC was 0.14 µg a.s./l.

Growth inhibition in Algae
The algastatic activity of fipronil was measured in a 96-h laboratory study using *Scenedesmus subspicatus*. No effect on biomass production, growth rate or appearance of the alga cultures was observed at test concentrations of 10, 20 and 40 µg/l. However, effects on both biomass production and maximum growth rate as well as abnormal appearance (colour and shape) of the algae were observed at 80 and 160µg a.s./l. The E_rC_{50} and E_bC_{50} were determined to be 74 µg a.s./l and 68µg a.s./l respectively. The NOE_rC was 40 µg a.s./l.

Inhibition to microbiological activity
The effects of fipronil on the microbiological activity in an aquatic medium with a mixed inoculum of microorganisms were studied in an activated sludge respiration inhibition test under laboratory conditions. The test substance was a straight formulation of fipronil containing 800 g fipronil/kg. After 3-h exposure, the respiration rate of the activated sludged was similar in the negative control and all the fipronil concentrations (ranging from 10 to 1000 mg a.s./l). As the activity of activated sludge was not affected at the highest concentration tested, the toxicity values derived from this study are: 3-h EC_{50} > 1000 mg a.s./l and NOEC= 1000 mg a.s./l.

Bioconcentration
The bioconcentration factor and bioaccumulation potential of [14C]-labelled fipronil were measured in a fish species (bluegill, *Lepomis macrochirus*). The test comprised an uptake phase (continuous flow-through over 35 days) and a depuration stage (14 days continuous flow-through in untreated medium). The uptake kinetics were considered to approach a simple 2-compartment model with measured BCF at steady state close to theoretical values predicted based on the log P_{ow}. The bioconcentration factor (BCF) estimated in whole fish was 321. Uptake residues were rapidly and nearly completely (> 99%) eliminated from whole fish within the 14-day depuration phase. The results of this study indicate no concerns of bioaccumulation of fipronil in aquatic animals.

Effects on aquatic organisms, further studies

Chronic toxicity to an appropriate species of fish
The chronic toxicity of fipronil to rainbow trout (*Oncorhynchus mykiss*) was investigated during an early life-stage exposure in a 90-day (60-day post hatch) flow through test. The LOEC for this study was determined to be 26 µg a.s./l and the NOEC was 15 µg a.s./l.

Chronic toxicity to appropriate invertebrate species
The chronic toxicity of fipronil to neonate *Daphnia magna* (< 24 hours old) was studied in a 21-day flow through test in the laboratory. The NOEC was determined to be 9.8 µg a.s./l based on mean body length and 20 µg a.s./l based of survival and reproductive performance.

Since aquatic insects are more sensitive to fipronil than daphnids, chronic testing has also been carried out on insect species. The toxicity of ^{14}C Fipronil to the larvae of the midge species *Chironomus riparius* was investigated in a water/sediment system in the laboratory where the test substance was applied to the overlying water. This was a static test with an exposure duration of 28 days. Based on initial measured concentrations in overlying water, the LOEC was 0.234 µg a.s./l and the NOEC was 0.117 µg a.s./l.

Effects on sediment dwelling organisms
The toxicity of sediment-applied fipronil to larvae of the midge species *Chironomus tentans* was determined in a long-term (10 days of exposure) spiked-sediment laboratory test. The 10-day LC50 was 30 µg a.s./kg and the NOEC was 16 µg a.s./kg. Although there are other long-term studies with *Chironumus* and fipronil, this result is the most relevant one because this it is the only test where the test substance was directly mixed into the sediment.

Aquatic plant toxicity
The toxicity of fipronil to the freshwater macrophyte *Lemna gibba* was studied in a 14-day static limit test in the laboratoy. There were no effects at the concentration tested. Therefore, the 14-d EC50 of fipronil in *Lemna gibba* is > 160 µg a.s./l and the 14-d NOEC is ≥ 160 µg a.s./l.

Effects on terrestrial organisms

Effects on soil micro-organisms
The effects of fipronil on the inhibition of the microbiological activity in natural soil was studied in the laboratory following exposure for 28 days. There was no adverse effects on soil respiration or nitrogen turnover at the two rates tested, which were equivalent to soil concentrations of 0.133 and 0.667 mg a.s./kg soil. Therefore, the NOEC (inhibition effects below 25%) was 0.677 mg a.s./kg soil.

Acute toxicity to earthworms
The acute toxicity of fipronil to earthworms (*Eisenia foetida*) was measured in a laboratory study conducted over 14 days in artificial soil according to the OECD Guideline No 207. The LC_{50} of fipronil was found to be greater than 1000 mg/kg. The NOEC was 1000 mg a.s./kg on the basis that no significant mortalities or adverse effects were observed after 14 days exposure.

Chronic toxicity to earthworms
The effects on reproduction and chronic toxicity of fipronil to earthworms (*Eisenia foetida*) were measured in a study conducted over 8 weeks of exposure in artificial soil. No adverse effects on survival, growth or reproduction were observed at any of the concentrations tested. The NOEC from this study was 1000 mg a.s./kg soil, the highest concentration tested.

The results of the acute and chronic tests indicate that fipronil is practically non-toxic to earthworms.

Effects on non-target arthropods – Toxicity to non-target soil-dwelling arthropods
An aged residue study was carried out to determine the duration of effects of Fipronil on the soil-dwelling arthropod beetle *Aleochara bilineata*. Soil was applied under field conditions at rates corresponding to nominal soil concentrations of 0.625, 1.25 and 2.5 mg a.s./kg soil. In total, 7 bioassays were carried out exposing *A. bilineata* to fresh and aged soil samples. One bioassay started directly after application (DAT 0) and further started 4,10,15,20,25 and 30 weeks after application (WAA). In parallel an analytical study was conducted to determine the actual concentration of fipronil and metabolites in the soil samples taken for the bioassays with *A. bilineata*. Based on the results of all bioassays and on measured concentrations of fipronil and major metabolites in soil, acceptable effects were observed at a concentration corresponding to 0.243 mg total residues/ kg soil.

Effects on birds - Acute oral toxicity
The acute oral toxicity of fipronil to bobwhite quail *(Colinius viginanus)* was measured in a 21-d LD_{50} study following exposure to single dosing. The LD_{50} of fipronil in bobwhite quail was determined at 11.3 mg ai/kg.

Effects on birds - Short term toxicity
The short-term dietary toxicity of fipronil to bobwhite quail *(Colinius viginanus)* was measured in a 22-d LC_{50} study. The LC50 was determined to be 48.0 mg a.s./kg diet and the NOEL was 19.5 mg a.s./kg diet.

Effects on birds - Effects on reproduction
The chronic toxicity of fipronil on the adults and reproduction performance of the bobwhite quail *(Colinius viginanus)* was measured in a 22-week dietary administration test in the laboratory. The study did not found direct adverse effects on reproductive paramenters but parental toxicity. Based on the overall results of the study, the NOEC was 10 mg a.s./kg diet.

Effects on terrestrial plants
The effects of fipronil (applied as a straight formulation containing 800 g fipronil/kg) on the seedling emergence of six species of plants (3 monocots and 3 dicots) were studied under greenhouse conditions. The test substance was mixed into the soil and then seeds of the six species were planted into the treated soil. The duration of exposure ranged from 21 to 25 days depending of the species. There were no adverse effects on seedling emergence and phytotoxicity at any of the soil concentrations tested (0.125, 0.5 and 2.0 mg a.s./kg soil). Plant weight at the end of exposure was not affected in four species, for which the NOEC is therefoe 2.0 mg a.s./kg. In two species (oilseed rape and oats) there was a reduction in plant weight at the highest concentration. Based on the results of these two species, the overall NOEC of the study is 0.5 mg a.s./kg soil.

Effects on Honeybees - Acute toxicity
The acute oral and contact toxicity of fipronil to honey bees (*Apis mellifera*) was studies in the laboratory. The acute oral and contact LD50 determined were 0.00417 and 0.00593 µg a.s./bee, respectively.

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Acceptability	**For the RMS corrected version, please refer to the document IIA Section 4.**
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Section A8 **Annex Point IIA, VIII**	**Measures necessary to protect man, animals and the environment**

Subsection (Annex point)				**Official use only**
8.1	**Recommended methods and precautions concerning handling, use, storage, transport or fire (IIA, VIII 8.1)**			
	Precautions to be taken in handling and storing:	**Handling** When using do not eat, drink or smoke **Storage** Keep out of the reach of children Keep away from food, drink and animal feedingstuffs Keep out of the light		
	Main Hazards	**Human health hazards** Toxic by inhalation, contact with the skin an by ingestion Danger of serious damage to health by prolonged exposure if swallowed **Environmental hazards** Very toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment		
	Exposure control/ Personal protection:	**Engineering measures** Keep the product as confined as possible. Capture dust at the point of emission **Hygiene measures** Keep the work place and equipment clean **Personal protective equipment** Filter dust mask, ventilated hood or ventilated coverall depending on exposure Wear gloves, coveralls and glasses		
	Transport:	**Transport Label; Solid pesticide, toxic**		
		RID/ADR Classe: 6.1	UNN°: 2588	Label 6.1
		MARITIME Classe: 6.1	UNN°: 2588 Page IMDG:6221	Marine Pollutant : Yes Label 6.1
		AIR Classe: 6.1	UNN°: 2588 Labelling group: III	Label 6.1
	Flash Point:	Not applicable		
	Extinguishing Media:	Water, foam, carbon dioxide or powder		
	Special Fire fighting Procedures:	Limit the spread of extinguishing media Wear suitable respiratory equipment		
	Usual Fire and Explosion Hazards:	Combustible, risk of toxic gases in the fumes		

	Reactivity Data:	No dangerous reactions known under the normal conditions of use		
	Stability:	Stable under normal conditions		
	Conditions to avoid:	None		
	Incompatibility (Materials to Avoid):	None		
	Hazardous Decomposition Products:	None		
	Hazardous Polymerization:	None		
	Conditions to avoid:	None		
	Steps to be taken in case material is released or spilled:	**Personal protection:** Filter dust mask, ventilated hood or ventilated coverall depending on exposure. Wear gloves, coveralls and glasses. **Environmental precautions:** Use appropriate containment to avoid environmental contamination. **Spillages:** Recover the product by dampening and sweeping up or by vacuum. The material and its container must be disposed of as hazardous waste.		
	Other Precautions:	None		
	Regulatory Information:	**Labelling:**		X
		R phrases:	23/24/25-48/25-50/53	
		S phrases:	(1/2-)-13-20/21-36/37-45-60-61	X
8.2	**In case of fire, nature of reaction products, combustion gases, etc. (IIA, VIII 8.2)**			
		Oxides of carbon, sulphur and nitrogen, hydrochloric and hydrofluoric acid.		
8.3	**Emergency measures in case of an accident (IIA, VIII 8.3)**			
8.3.1	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment, if available**	**First aid measures:** **Inhalation**: Keep patient calm, remove to fresh air, seek medical attention. **Ingestion:** Rinse mouth immediately and then drink plenty of water, seek medical attention. Do not induce vomiting unless told by a poison control center or doctor. Never induce vomiting or give anything by mouth if the victim is unconscious or having convulsions. **Skin contact:** Immediately wash thoroughly with soap and water, seek medical attention. **Eye contact:** Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.		

8.3.2	**Emergency measures to protect the environment**	Recover the product by dampening and sweeping up or by vacuum. Use appropriate containment to avid environmental contamination. The material and its container must be disposed of as hazardous waste.	
8.4	**Possibility of destruction or decontamination following release in or on the following: (a) air, (b) water, including drinking water, and (c) soil (IIA, VIII 8.4)**		
		The active substance Fipronil is a solid with a very low vapour pressure. A contamination of the environmental compartment air is therefore unlikely after the release of Fipronil into the environment due to an accidental misuse. Recover the product by dampening and sweeping up or by vacuum. Use appropriate containment to avoid environmental contamination. The material and its container must be disposed of as hazardous waste.	X
8.5	**Procedures for waste management of the active substance for industry or professional users (IIA, VIII 8.5)**		
8.5.1	**Possibility of re-use or recycling (IIA, VIII 8.5.1)**	**Product disposal:** Dispose of this material and its container at hazardous or special waste collection point. Incinerate at a specialist facility – minimum temperature 1150°C minimum residence time 2 seconds. **Container disposal:** Commercial containers must be completely empty. As for product/container.	
8.5.2	**Possibility of neutralisation of effects (IIA, VIII 8.5.2)**	Please refer to the disposal considerations given above.	
8.5.3	**Conditions for controlled discharge including leachate qualities on disposal (IIA, VIII 8.5.3)**	Please refer to the disposal considerations given above.	
8.5.4	**Conditions for controlled incineration (IIA, VIII 8.5.4)**	Please refer to the disposal considerations given above.	
8.6	**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIA, VIII 8.6)**		
		Very toxic to aquatic organisms	X
8.7	**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances (IIIA, VIII 1)**		

	Organohalogen compounds are covered by List I of the Annex to Directive 80/68/EEC. Biocides and their derivatives are covered by List II of the Annex to Directive 80/68/EEC.	**X**

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Adopt applicant's version with following amendment : **8.1 Recommended methods and precautions concerning handling, use, storage, transport or fire** Regulatory Information: ***R phrases:*** *23/24/25-48/25-50/53* ***S phrases:*** *(1/2-)-13-20/21-36/37-45-60-61* Not required in this part, see the document IIIA 9. **8.4 Possibility of destruction or decontamination following release** *The active substance Fipronil is a solid with a very low vapour pressure. A contamination of the environmental compartment air is therefore unlikely after the release of Fipronil into the environment due to an accidental misuse.* There is no decontamination procedures available. The product should be kept away from water and soil *Recover the product by dampening and sweeping up or by vacuum.* *Use appropriate containment to avoid environmental contamination.* *The material and its container must be disposed of as hazardous waste.* **8.6 Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms (IIA, VIII 8.6)** *Very toxic to aquatic organisms* Very toxic to bees and fauna **8.7 Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances (IIIA, VIII 1)** Fipronil and its derivates, as o*rganohalogen compounds, are covered by List I of the Annex to Directive 80/68/EEC.* Firponil, as *Biocides, and ~~their~~* its *derivatives are covered by List II of the Annex to Directive 80/68/EEC.*
Conclusion	Adopt applicant's version
Reliability	Not applicable (no study or test data)
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	**in or on the following: (a) air, (b) water, including drinking water, and (c) soil (IIA, VIII 8.4)**
Results and discussion	

Conclusion
Reliability
Acceptability
Remarks

Section A9 **Annex Point IIA, IX**	**Classification and labelling**

Classification:	as in Directive 67/548/EEC*		
Hazard symbols:			
Indication of danger:	T (Toxic) N (Dangerous for the environment)		
Risk phrases:	23/24/25	Toxic by inhalation, in contact with skin and if swallowed	X
	48/25	Toxic: danger of serious damage to health by prolonged exposure in contact with skin	
	50/53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment	
	55	Toxic to fauna	X
	57	Toxic to bees	X
Safety phrases:	45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)	X
	60	This material and its container must be disposed of as hazardous waste	
	61	Avoid release to the environment. Refer to special instructions/safety data sheet	

* In September 2004, the EU Commission Working Group "Technical Committee on Classification and Labelling of Dangerous Substances" concluded Expert discussions on Classification and Labelling of Fipronil for the areas "human health" and "environment". The decision on classification and labelling of Fipronil is to be endorsed in the 30th ATP of Directive 67/548/EEC

Justification for proposed classification	T; R 23/24/25 These R-phrases derive from the result of the studies with the active ingredient on acute oral and inhalation toxicity in rats, and on acute dermal toxicity in rabbits. T; R 48/25 The R-phrase "Toxic: Danger of serious damage to health by prolonged exposure if swallowed" is based on clinical symptoms of neurotoxicity and substance-related deaths in repeated-dose toxicity studies. N; R 50/53 The classification is proposed on the basis that the LC_{50} (fish, 96 h), the EC_{50} (daphnia, 48 h) and the IC_{50} (algae, 72 h) are less than 1 mg/l and that the substance is not readily biodegradable.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2007
Risk phrases	Agree with applicant's version. **Revisions/amendments:** *48/25 Toxic: danger of serious damage to health by prolonged exposure ~~in contact with skin~~* by ingestion ~~*55 Toxic to fauna*~~ ~~*57 Toxic to bees*~~ For the moment, there are no clear criteria to give this type of R-phrase to a molecule. Moreover these phrases have not been proposed for the 30th ATP of the 67/548/EC directive. These phrases have been proposed for the 30th ATP of the 67/548/EC directive: S1/2 Keep locked up and out of the reach of children S28 After contact with skin, wash immediately with plenty of water S36/37 Wear suitable protective clothing and gloves

Classification according to CLP regulation	AcuteTox. 3; STOT Rep.1 Aquatic acute 1 Aquatic chronic 1
Hazard Statement	H301: Toxic if swallowed H311: Toxic in contact with skin H331: Toxic if inhaled H372: Causes damage to organs (central nervous system) through prolonged or repeated exposure H400 Toxic to aquatic life H410 very toxic to aquatic life with long lasting effects M-factor : 1000

Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A10 **Annex Point IIA, IIIA**	**Summary and Evaluation of Sections 2 to 9** **(RMS: This section A10 was not evaluated because it was the same document than the document IIA. Thus, see this document to have the corrections of this section too.)**

10.1 Section A2 Identity

10.1.1 Identification of the substance

CAS-No.120068-37-3

CIPAC No. 581

EU No : 424-610-5

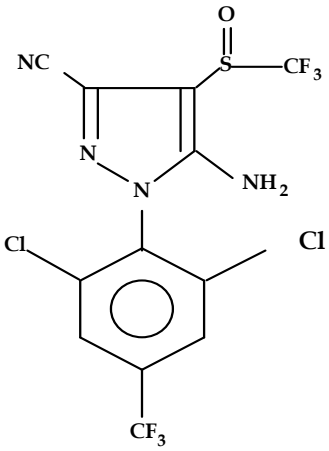
IUPAC Name : (±)-5-amino-1-(2,6-dichloro-α,αα,-trifluoro-p-tolyl)-4-trifluoromethylsulfinyl-pyrazole-3-carbonitrile

CAS name: 5-amino-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile

Common name: Fipronil

Molecular formula: $C_{12}H_4Cl_2F_6N_4OS_3$

Structural formula



Molecular weight : 437.15

Purity : Technical material 95%w/w minimum

10.2 Section A3 Physical and Chemical Properties

Fipronil is a white crstaline solid at room temperature. Its melting boint is ca 200°C and it decomposes before reaching its boiling point. The relative density of pure fipronil is $D^R_4 = 1.705$ and its vapour pressure is 3.7×10^{-7} Pa at 25°C.

The Henry's constant at 25°C using data from purified material was calculated to be:
2.31×10^{-4} $pa.m^3.mol^{-1}$. Fipronil has low solubility in water (3.78 mg/l at 20°C; pH: 6.58) and most organic solvents (except acetone 5.459g/l).

Partition coefficient - n-octanol/water for the purified material is 3.5 – 4.0 at 20°C.

Fipronil is neither flammable nor auto-flammable nor does it degrade at temperatures at temperatures below its melting point (>200°C). Fipronil has no oxidising or explosive properties and shows no re-activity towards its container material (polythene).

10.3 Section A4 Analytical Methods for Detection and Identification

Fully validated analytical methods with acceptable levels for linearity, specificity and recovery and with appropriate limits of detection (where applicable) are availale for the following :

The purity of technical material (HPLC-UV)
The levels of impurities in technical material
Residues of fipronil and its metabolites in soil (GC –EC/MS or LC-MS/MS)
Residues of fipronil and its metabolites in drinking water (GC-MS or GC-EC)
Residues of fipronil and its metabolites in surface water (LC-MS/MSor GC-EC)
Fiproles in blood plasma (GC-EC)
Residues of fipronil and its metabolites in plants (GC-ES/).
Residues of fipronil and its metabolites in foodstuffs of animal origin (LC-MS/MS)

10.4 Section A5 Effectiveness against Target Organisms and Intended Uses

10.4.1 Function

Insecticide

10.4.2 Field of use envisaged

Cockroaches

10.4.3 Effects on target organisms

Fipronil affects the nervous system of insects. It has both contact and ingestion activity but is particularly effective by way of ingestion.
Fipronil interferes with the passage of chloride ions through the Gamma Aminobutyric Acid (GABA) chloride channel, disturbing the central nervous system and causing subsequent insect death. It can be noted that fipronil displays a tighter binding to the insect GABA chloride channel than to vertebrate, particularly mammals, providing useful selective toxicity.

10.5 Section A6 Toxicological and Metabolic Studies

Toxicokinetic studies on the absorption, distribution, metabolism and excretion of Fipronil have been investigated in the Sprague-Dawley rat following either single oral treatment with radiolabelled fipronil (4 and 150 mg/kg bw) or by use of a multiple dosing regime (i.e. a single oral gavage administration of radiolabelled fipronil after 14-day treatment with daily oral doses of non-radiolabelled fipronil at a dose of 4 mg/kg bw/d). Additionally the blood, tissue and bile kinetics of ^{14}C-Fipronil have been investigated at the dose rates of 4 and 40 mg/kg bw.

Oral absorption
At low oral dose levels fipronil is rapidly absorbed from the gastrointestinal tract with peak blood levels (mean 0.55 µg eq./g) achieved after 5-6 hours post dosing with 4 mg/kg bw. After administration of 40 or 150 mg/kg bw, peak blood levels occurred after 34-38 or 48-58 hours, respectively. Maximum blood concentrations were proportional to the administered dose level.

Since absorbed Fipronil is mainly excreted via the bile, the gastrointestinal absorption of an orally administered low dose was estimated based on the results of a bile excretion experiment in rats. An oral absorption estimate of about 90% was estimated from radiolabel recoveries in urine, bile and tissues within 72 hours following oral gavage treatment with 4 mg/kg bw radiolabelled Fipronil.

Distribution
Distribution of fipronil is rapid after low-dose oral treatment and delayed after administration of high doses. Fipronil is widely distributed following oral administration. Radiolabelled residues were predominantly detected in fatty tissues. The fat showed the highest tissue levels (4 mg/kg bw: 31 µg eq./g at 4.8 h; 40 mg/kg bw: 229 µg eq./g at 33.6 h), which were reduced seven days after treatment (4 mg/kg bw: 16–23 µg eq./g; 40 mg/kg bw: 32–39 µg eq./g).

In the liver, peak concentrations of approx. 10 µg eq/g seen within the first hour after treatment with 4 mg/kg bw were reduced to approx. 2.7 µg eq/g within 7 days. After administration of 40 mg/kg bw, mean liver residue levels were in the range of 31-36 µg eq./g during 3–40 h post dosing, decreasing to about 6 µg eq./g after seven days.

Moderate levels were found in the adrenals, pancreas, skin, liver, kidney, ovaries, uterus and muscle.

In the brain, residue levels of about 1.8–2.4 µg eq/g were reached within 1 hour of single treatment with 4 mg/kg bw, decreasing to about 1 µg eq/g after seven days. High-dose treatment resulted in brain residue levels of about 9 µg eq/g 3-4 hours after dosing, decreasing to about 1.6–2 µg eq/g after seven days.

Metabolism

Fipronil is intensively metabolised with no free parent material being detected in either the urine or tissues. No obvious dose-level-, dose-regimen- or sex-dependent differences were observed. In the **faeces**, at least 11 radiolabelled metabolites were found. At early time points of investigation, the parent Fipronil and lesser amounts of M&B 46136, M&B 45950 and RPA 200766 were predominantly detectable; at later time points, the sulfone metabolite M&B 46136 was the major metabolite in faeces. In **bile**, a minimum of 16 components were observed, of which Fipronil, M&B 46136, M&B 45897 and RPA 200761 were identified. Evidence from the faecal metabolite profiles suggests that these conjugates are subject to further biotransformation in the gut before elimination in the faeces. HPLC analysis of **urine** indicated the presence of very polar material that was found to consist of 18 components after the urine samples were subjected to enzymatic hydrolysis by glucuronidase and sulphatase enzymes. The two major metabolites in urine following deconjugation were found to be ring-opened pyrazole products while Fipronil (M&B 46030), RPA 200766 were also observed. All these components were thought to have been present as N-glucuronides in the untreated urine. In the **tissues** that were examined for their metabolic profile (fat, liver, kidney, muscle and uterus), one major radiolabelled component was detected and identified to be M&B 46136. A proposed metabolic pathway in rats is presented below.

Figure 10.5-1 Proposed metabolic profile of fipronil in the rat

XXXX

Elimination

After single oral gavage administration of a low dose of radiolabelled fipronil (4 mg/kg bw), approx. 46% of the administered radiolabel was recovered in the faeces, approx. 6% of the dose was eliminated via the urine and a further 1% was recovered in the cage wash, within a 7-day post-treatment period. Higher levels of elimination were observed after daily administration of 4 mg/kg bw/d unlabelled fipronil followed by a single radiolabelled fipronil low dose, where 7-day recoveries in urine, faeces and cage wash amounted to 16%, 56%, and 1.6% of the administered radiolabel in males and 14%, 61% and 3% in females, respectively. Within 7 days after single oral gavage administration of 150 mg/kg bw/d, mean radiolabel recoveries in urine were about 29% and 22% of the administered dose in male and female group rats, respectively, in faeces about 67% and 75%, and in cage wash about 4.5% and 4% of the administered dose in males and females, respectively. Elimination in the expired air was shown not to be a significant route of excretion.

In the biliary excretion study in rats, recovery of a single low fipronil dose (4 mg/kg bw) was about 7% in the bile, 10–14% in faeces and 1–2% in urine & cage wash within 3 days after treatment. After single high-dose treatment (40 mg/kg bw), mean radiolabel recovery within 3 days in the male group was about 25% in bile, 6% in urine & cage wash, and 21% in faeces; radiolabel recoveries in the female group were about 12% in bile, 4% in urine & cage wash and 27% in faeces.

Dermal absorption

The in vivo dermal absorption study in male rats (using radiolabelled Fipronil formulated in Regent 80 WDG = BAS 350 00 I), has demonstrated that less than 1% (<0.005 to 0.65%) of the applied Fipronil dose was absorbed following 10 hours of continuous exposure to three different test concentrations. At this time period less than 2% of the applied dose (0.69-1.87%) was found at the treated skin site after washing. Despite the low percentage of the dose found to be absorbed in the rat in vivo study, the comparative in vitro data demonstrates that the use of the rat as a model for humans significantly overestimates the percent absorbed. Furthermore, results from *in vitro* dermal absorption studies on human skin with a range of formulations covering different properties and vehicles (25 ULV, 300 EC, 50 SC, and 200 SC) indicate that the percent of the applied dose absorbed does not significantly vary with formulation type. Consequently the nature of the vehicles and components used in various formulation types does not appear to seriously impact the dermal absorption factor of Fipronil in humans.

Table 10.5.1 Summary of results from dermal absorption studies with fipronil formulations

Fipronil Formulation (nominal Fipronil test concentration)	% absorption (rat / human)		Recovery in/on application site (rat / human)		Flux (µg/cm/h) (rat / human)		Flux ratio (rat / human)
In-vivo rat (10-h exposure)							
Regent 80 WDG (9 g/l)*	0.65	n/a	1.87	n/a	n/a	n/a	n/a
In-vitro rat / human (24-h exposure)							
25 ULV (25 g/l) **	4.3	0.32	4.14	2.64	0.45	0.037	12
300 EC (300 g/l)**	0.82	0.15	7.0	1.07	1.03	0.215	5
50 SC (50 g/l)**	15	0.15	7.09	0.95	2.99	0.052	58
200 SC (200 g/l)**	1.19	0.05	No data	No data	9.92	0.42	24

* Data from lowest test concentration (0.9% w/v as carboxymethyl cellulose suspension)
** Data from undiluted products

Thus, both in vitro and in vivo methods of assessment demonstrate that Fipronil has a low potential for dermal absorption. Absorption in humans is expected to be considerably less than 1% of the total dermal exposure. Consequently, the use of a 1% absorption factor following dermal exposures to Fipronil is deemed to be conservative and appropriate to be used in human risk assessments.

From a tissue kinetic study in the rat it is concluded that after a single oral administration of (14C)-Fipronil to male and female rats at the nominal dose levels of 4 and 40 mg/kg body weight, the rate absorption of radioactivity appeared to be independent of sex, but dependent upon dose level with a rapid absorption being observed for the 4 mg/kg dose group but a slower absorption rate being observed after administration at the 40 mg/kg dose level. Radioactivity was widely distributed in the tissues with predominance in fatty tissues. The relatively long elimination half-lives suggest the presence of a deep compartment such as fat.

Acute toxicity:

Fipronil was moderately toxic following acute exposure. The acute oral LD_{50} to male rats was 92 mg/kg bw in males, 103 mg/kg bw in females and 97 mg/kg bw for both sexes combined. Similar acute oral LD_{50} values were obtained in mice (98 / 91 / 95 mg/kg bw in males / females / combined sexes, respectively). Under the experimental conditions employed, the acute dermal toxicity in rats was low (LD_{50} > 2000 mg/kg bw), while in rabbits mortality occurred at dose levels of 250 mg/kg bw and above resulting in an acute dermal LD_{50} of 445 mg/kg bw in males and 354 mg/kg in females. Two acute 4-hour nose-only inhalation toxicity studies were conducted. The LC_{50} values for males and females combined were 0.68 mg/l for unmilled dry technical material. When Fipronil was air-milled to meet USEPA particle size requirements for inhalation testing, Fipronil LC_{50} values of 0.36 mg/l and 0.42 mg/l were obtained for male and female rats, respectively.

Clinical signs seen following acute oral and inhalation exposure were consistent with those anticipated following administration of a chemical interacting with a neurotransmitter receptor i.e. a combination of abnormal (waddling) gait, hunched posture, piloerection, lethargy, tremors and convulsions. The combination was seen at dose levels within the lethal range. Convulsions, which were repeated in some animals, were not seen at non-lethal dose levels. Similar signs (convulsions, spasms, tremors) as well as perinasal staining and emaciation were seen in rabbits following acute dermal treatment.

Fipronil was not a skin or eye irritant according to EU classification criteria nor was it a skin contact sensitizer in a Magnusson and Kligman Maximisation or in a Buehler tests.

Repeat dose toxicity:

Short term oral studies in rats

In the 28-day study, one female rat given the highest dose level, 400 ppm, died in Week 1. Its death may or may not have been treatment-related. A transient dose-related loss of bodyweight occurred on Day 5 at 100, 200 and 400 ppm which was considered to be due to an initial reduction in food consumption. Thyroid follicular cell hypertrophy was seen in all treated groups and hepatocyte hypertrophy in the liver was found at 100 ppm and above, apart from in females where there were no hepatic changes at 100 ppm. These findings were associated with increases in bodyweight-relative liver weight at 100 ppm and above and marginally higher bodyweight-relative thyroid weights in all groups of treated females. Clinical chemistry findings were increased total protein and globulin in all treated groups, although there was no dose-related response, high cholesterol in both sexes at 400 ppm and in females fed 25 and 200 ppm, and decreased albumin in females at 400 ppm. The only hematological finding was an increase in platelets at 200 and 400 ppm. Based on histopathological findings in the liver and thyroid, a No Observed Effect Level (NOEL) was not identified in this study i.e. NOEL < 25 ppm which corresponded to 3.4 mg/kg bw/d in males and 3.5 mg/kg bw/d in females.

Dose levels of 0, 1, 5, 30 and 300 ppm were used in the 90-day rat study. A single incident of clinic convulsion in one high dose level male was noted in Week 9. At the highest dose level, a transient reduction in food intake and body weight gain was seen during the first week of treatment. Male body weight gain at this dose level was still reduced during Week 2. A dose-related increase in liver weight at 5 ppm and above, together with changes in plasma aminotransferase activity, protein, urea and glucose in treated rats, was considered indicative of altered liver function. At dose levels of up to 5 ppm, plasma changes were generally minor and not associated with any histopathological change. Therefore, they were considered to be adaptive metabolic responses to the administration of a xenobiotic and not likely to be of direct toxicological significance. At 300 ppm, a degree of toxicity was induced, manifest as increased in fat deposition in males. A minor disturbance of red cell parameters was noted in females from this dose level. Thyroid follicular hypertrophy and thyroid hyperplasia was also seen at 300 ppm and was probably indicative of a perturbation of the hypothalamo-pituitary-thyroid-liver axis resulting from the increased metabolic activity of the liver. The study report also concluded that the tendency for increases in liver and thyroid weights and

changes in serum protein levels observed at 30 ppm were not toxicologically relevant in the absence of histopathological changes. However, given the observations of mild thyroid changes at 25 ppm in the 28-day oral toxicity study in rats the dose level of 30 ppm is considered to represent a Low Observed Adverse Effect Level (LOAEL) in this study. The No Observed Adverse Effect Level (NOAEL) was established at 5 ppm, which corresponded to 0.33 and 0.37 mg/kg bw/d in male and female rats, respectively.

Short term oral studies in dogs

The 90-day dog study used dose levels of 0, 0.5, 2 and 10 mg/kg bw/d. Overt toxicity was seen at 10 mg/kg bw/d since three animals were killed for humane reasons within the first 2 weeks of treatment following convulsive episodes. Clinical signs of general toxicity included inappetence, underactivity, hunched posture and emaciation which were particularly marked during the first two or three weeks of treatment. Tremors and/or convulsions and/or head nodding were indicative of neurological disturbance. Similar findings, as well as facial twitching and ataxia in one female were seen during veterinary examinations and exaggerated blink and gag reflexes and depressed tactile placing responses were noted during neurological evaluations. Bodyweight loss or stasis occurred in Week 1 at 10 mg/kg bw/d but the surviving male recovered. Overall bodyweight gain in the other dogs at this dose level was similar to controls. At 2 mg/kg bw/d, transitory weight loss or stasis was seen in two females. Food consumption was markedly lower at 10 mg/kg bw/d during Week 1 with inappetence from Day 1 but, again at 2 mg/kg bw/d, only two females showed inappetence during Week 2. These findings were associated with high alkaline phosphatase and low cholesterol activity in males given 10 mg/kg bw/d. There were no direct treatment-related histopathological changes. The follicular and parafollicular atrophy of the mesenteric lymph nodes and cortical atrophy of the thymus in one male and cortical atrophy of the thymus in one female at 10 mg/kg bw/d were considered to be due to stress rather than a direct effect of Fipronil. Based on the slight inappetence and depression of bodyweight in females at 2 mg/kg bw/d, the No Observed Effect Level (NOEL) was 0.5 mg/kg bw/d.

Two one-year studies were conducted in dogs with dose levels of 0, 0.2, 2 and 5 mg/kg bw/d in one study and with 0, 0.075, 0.3, 1 and 3/2 mg/kg bw/d in the other. Following significant toxicity, the highest dose in the second study was reduced to 2 mg/kg bw/d after 38 days of treatment. In both studies, clinical signs indicative of neurological disturbance were seen at dose levels of 1 to 5 mg/kg bw/d and included convulsions, twitching or tremors, changes in behaviour (nervousness or aggression) and activity patterns, and gait abnormalities. Other signs were exaggerated rigidity or stiffness of limbs, ataxia, vocalisation, head nodding and resistance to dosing. Although the signs at 2 and 5 mg/kg bw/d were confirmed by veterinary and specific neurological examinations, no similar confirmation was obtained in the second study at 3/2 mg/kg bw/d, apart from one male which showed extensor flexor withdrawal. No clinical signs were seen at 0.3 mg/kg bw/d or below. Transitory periods of food inappetence were seen at 5 mg/kg bw/d in dogs showing clinical signs but only one male showed reduced body weight gain. There were no other treatment-related effects, including no histopathological changes. The No Observed Adverse Effect Level (NOAEL) in the first study was 0.2 mg/kg bw/d and the No Observed Effect Level (NOEL) in the second study was 0.3 mg/kg bw/d.

Short-term dermal toxicity study in rabbits

In a 21-day dermal toxicity study one male and one female rabbit given the highest Fipronil dose level, 10 mg/kg bw/d, exhibited a period of extreme hyperactivity near the end of the study. Both recovered however, the findings in the present study were considered to be treatment-related. Overall bodyweight gain was significantly reduced in males at this dose level. Therefore the No Observed Effect Level (NOEL) was 5 mg/kg bw/d.

Genotoxicity/mutagenicity:

Fipronil was tested in a battery of *in-vitro* and *in-vivo* assays measuring several different endpoints of potential genotoxicity such as gene mutation, chromosomal aberration and DNA damage/repair [see Table 6.18/5]. Results from mutagenicity studies indicated that Fipronil does not induce base pair substitutions or frame-shift mutations in any of the bacterial tester strains, or gene mutation in mammalian cells in culture. Since the original tests did not include the bacterial test strain TA 102 or E. coli WP2 uvrA, a supplementary Ames test was conducted with E.coli WP2 uvrA, which confirmed the absence of bacterial mutagenicity of Fipronil. Furthermore, there was no evidence of activity in the in vitro mammalian cell gene mutation assay with Chinese hamster V79 cells evaluating forward mutation at the HGPRT locus. In the chromosomal aberration test in CHL cells, a positive result was obtained in both the absence and presence of S9 mix following 6 hours of exposure, however only in the presence of substantial cytotoxicity. The positive response was not reproducible in the same cell system after 24- and 48-hour exposure periods. A second in vitro chromosomal aberration assay in human lymphocytes was also negative. Fipronil was demonstrated not to provoke a clastogenic response in two in vivo micronucleus assays. These studies were performed at dose levels of up to 25 and 50% of the oral LD_{50}, respectively, in which clear systemic toxicity was observed. In an in-vivo UDS assay in rat hepatocytes conducted to further substantiate the lack of concern for a genotoxic potential, Fipronil was tested at dose levels of up to 50% of the rat oral LD_{50}; this UDS test did not provide any evidence of DNA damage by Fipronil under the treatment conditions.

In conclusion, using an overall weight-of-evidence approach, Fipronil is not considered to be mutagenic or genotoxic. The only (questionable) positive response observed in one of two *in-vitro* chromosome aberration assays was not confirmed in two corresponding *in-vivo* assays that measure the same endpoint (mouse micronucleus test). In addition, a second *in-vivo* study (UDS test in Wistar rats) did not reveal any effects on the DNA.

Long-term toxicity/carcinogenicity:

Dietary long-term studies with Fipronil comprised a 104-week combined rat chronic toxicity and oncogenicity study and a 78-week mouse oncogenicity study. They were conducted between October 1990 and October 1992 in accordance with prevailing USEPA and EU testing guidelines.

Rat

In the combined chronic toxicity and oncogenicity study with rats, satellite groups for a 13-week 'off-dose' reversibility period after 52 weeks of treatment were included. These satellite animals were used to measure thyroid hormone levels during treatment and in the 'off-dose' Reversibility period. The duration of the study was intended to be 104 weeks, however, males from the Oncogenicity phase were killed after 89 weeks and females after 91 weeks when survival reached 25% (in high dose males and the 30 ppm females). The survival of the rat strain used was consistent with several other contemporary studies in Sprague Dawley CD rats. Survival was not affected by treatment in the absence of a dose-response relationship.

Convulsive episodes were observed in a dose dependent manner at 1.5 ppm and above. At the highest dose level, 300 ppm, they resulted in death in four males and three females and a slight increase in mortality at this dose level during the early part of the study. Neurological signs, including irritability, overactivity, vocalisation and aggressive behaviour, were seen at 300 ppm, especially in females, and to a lesser degree at 1.5 and 30 ppm. Food consumption and body weight gain was reduced at 300 ppm and 30 ppm. The clinical signs and the impaired body weight development indicated that the high-dose level of 300 ppm exceeded the Maximum Tolerated Dose (MTD).

Large livers and increased liver weights observed at 300 ppm were correlated with changes in plasma protein, cholesterol, and shorter prothrombin times, although no corresponding histopathological findings were noted in the liver. These findings, which persisted into the 13-week 'off-dose'

Reversibility period, were indicative of enhanced liver function, a common adaptive change to administration of a xenobiotic. Therefore, they were not of direct toxicological significance.

Increases in thyroid weights in males alone at 30 ppm and in both sexes at 300 ppm were associated with a dose-related imbalance of the thyroid / pituitary hormone axis. High circulating levels of thyroid stimulating hormone (TSH) occurred at 30 ppm and 300 ppm through Week 50. Although cessation of treatment during the Reversibility phase was followed by an immediate recovery in TSH levels in females at 300 ppm and in males at 30 ppm, while recovery in the high dose males was not complete by the end of this 'off-dose' period. Thyroxine (T_4) levels were reduced in all treated groups and were particularly marked at 300 ppm where it was undetectable during the first week of treatment. At 1.5 ppm and above, T_4 levels were decreased through Week 50 whilst at 0.5 ppm low values were recorded up to Week 24. During the Reversibility period, T_4 remained depressed in males at 30 and 300 ppm for the first two weeks but recovery was complete by Week 11. Levels of the more physiologically active triiodothyronine (T_3) were less affected although there was a significant decrease during the first week of treatment in males given 300 ppm.

This hormonal imbalance was associated with macroscopic and microscopic changes in the thyroid. Increased liver and thyroid weights were seen in both the chronic toxicity and oncogenicity phases of the study. Although absolute organ weights were comparable with controls at the end of the reversibility period, bodyweight-relative weights were still high at 300 ppm, reflecting the lower bodyweights of these animals. Increased thyroid weights corresponded with a higher incidence of follicular cell adenomas and carcinomas in oncogenicity phase rats fed 300 ppm. Incidences at lower dose levels were within the historical control range. No other treatment-related tumourigenic findings were observed.

Mechanistic investigations on thyroid tumourigenisis in the rat (summarised below) indicate that all of these thyroid responses were secondary to the enhanced metabolic activity of the liver, which increased the clearance of the plasma thyroid hormones thereby disturbing the negative feedback to the pituitary. This, in turn, resulted in increased release of TSH and stimulation of the thyroid. It also explains the presence of the increased incidence of thyroid follicular tumours at 300 ppm in the oncogenicity phase. When all animals from this phase are considered together, a significant increase in benign adenomas was found in both sexes at this dose level and an increase in malignant carcinomas in males alone. These thyroid follicular cell tumours arose as a result of an adaptive, enhanced metabolic activity of the liver i.e. via a non-genotoxic mechanism. The thyroid gland of rats is well known to be particularly sensitive to disturbances in thyroid hormone balance compared to the human thyroid. Consequently, this syndrome, including the follicular cell tumours, may have no relevance to the risk to humans from exposure to Fipronil.

A higher incidence and severity of progressive senile nephropathy seen at 30 and 300 ppm was a possible enhancement of this spontaneous age-related change by Fipronil. Elevated kidney weights and macroscopically enlarged and pale organs were a likely consequence of this finding. Higher urinary protein excretion was also likely to be related. However, the rationale for slightly high plasma calcium and low urinary pH at 30 and 300 ppm was unclear. Because there were no histopathological changes associated with high spleen and adrenal weights at 300 ppm, these weight changes were considered to be of no toxicological significance.

In conclusion, dietary administration of Fipronil to rats for their life span at up to 300 ppm produced a number of effects, particularly at 30 and 300 ppm. Functional and morphological changes were found in the liver, thyroid and kidney and functional effects alone in the central nervous system. At the highest dose level of 300 ppm, thyroid follicular cell tumours resulted from elevated TSH levels producing an intense stimulation of this gland.

The NOEL for thyroid tumours was established at 30 ppm. The overall No Observed Adverse Effect Level (NOAEL) was 0.5 ppm, corresponding to 0.019 mg/kg bw/d of Fipronil in males and 0.025 mg/kg bw/d in females.

Mouse
Dietary dose levels of 0, 0.1, 0.5, 10, 30 and 60 ppm of Fipronil were evaluated in the 78-week mouse oncogenicity study. Satellite groups at each dose level were killed after 53 weeks in order to assess chronic toxicity.

High mortality at 60 ppm during the first 9 weeks of treatment showed that this dose level exceeded the maximum tolerated dose. Consequently, it was terminated in Week 10. Mortality was comparable in other groups. Although a convulsion was observed prior to death in one male, no clinical signs were seen in other animals and there were no treatment-related lesions at necropsy. Convulsions were recorded in two other males at this 60 ppm dose level. Significantly low body weight gains were seen at 30 ppm. Low gain was also noted at 10 ppm for the first 13 or 26 weeks in males and females, respectively. Food consumption was consistently low at 30 ppm.

The liver was identified as the target organ. Increases in organ weights at 10 ppm (males alone) and 30 ppm (both sexes) were seen after 53 and 78 weeks. In the terminal killed males at 30 ppm these were correlated with liver enlargement and changes on the surface of the liver. Histopathologically, at both dose levels there was an increased incidence of periacinar microvesicular vacuolation in males after both 53 and 78 weeks and also at 0.5 ppm and above in females. In decedent males at 30 ppm, hepatocellular hyperplasia and chronic degenerative changes (including necrosis in occasional cells, apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes) were found. No evidence of oncogenicity was found in this study.

The No Observed Effect Level (NOEL) was 0.5 ppm, corresponding to 0.055 mg/kg bw/d of Fipronil in males and to 0.063 mg/kg bw/d in females.

Reproductive toxicity/developmental toxicity:
Developmental toxicity
In the rat prenatal toxicity study, dose levels of 0, 1, 4 or 20 mg/kg bw/d of Fipronil were administered by oral gavage to groups of 25 mated Sprague-Dawley (CD) rats from Days 6 to 15 *post coitum* (pc). Control animals were given the vehicle, 0.5% Methyl-cellulose only. The highest dose level, 20 mg/kg bw/d, was maternally toxic, as demonstrated by reduced bodyweight gain and food consumption from Day 6 to 10 pc. Bodyweight gain was 60% below controls during this period. Water consumption was slightly high from Days 8 to 20 pc. Marginally low weight gain was also observed at 4 mg/kg bw/d but was considered to be of doubtful biological significance. There was no effect upon litter parameters or on foetal weight. Moreover there were no treatment-related effects on foetal morphology. Fipronil was not teratogenic in the rat. The No Observed Adverse Effect Level (NOAEL) for maternal toxicity in the rat was 4 mg/kg bw/d and the No Observed Effect Level (NOEL) for developmental toxicity was 20 mg/kg bw/d.

In the rabbit prenatal toxicity study, dose levels of 0, 0.1, 0.2, 0.5 or 1.0 mg/kg bw/d were administered by oral gavage from Days 6 to 19 *post coitum* to groups of 22 mated New Zealand White rabbits. Control animals were given the vehicle, 0.5% methylcellulose and 0.5% Tween 80 alone. Dose levels of 0.5 and 1.0 mg/kg bw/d of Fipronil were maternally toxic, as demonstrated by reductions in bodyweight gain and food consumption throughout the treatment period. Marginally low bodyweight gains and food intakes at 0.1 and 0.2 mg/kg bw/d were considered to be of doubtful biological significance bearing in mind the inherent variability in rabbits.

There was no effect on litter parameters or on foetal and placental weight. Moreover there was no treatment-related effect on foetal morphology. Fipronil was not teratogenic in the rabbit.

The maternal No Observed Adverse Effect Level (NOAEL) was 0.2 mg/kg bw/d. The No Observed Effect Level for developmental toxicity was 1.0 mg/kg bw/d.

Reproductive toxicity
In a two-generation reproduction toxicity studies, Sprague-Dawley (CD) rats were fed dietary concentrations of 0, 3, 30 and 300 ppm of Fipronil continuously for two generations. Two litters were derived from the first (F_0) generation and a single litter from the second F_1 generation. At the 300 ppm high dose level, mortality, convulsions, reduced bodyweight gain and food consumption were seen in F_0 and F_1 adults. Mating performance of the F_1 generation was slightly reduced at this dose level (83% compared with 100% in controls) with a consequent reduction in fertility index. Increased liver and thyroid weights (absolute and bodyweight relative) were seen at both 30 and 300 ppm in adults from both generations. The increased liver weight at 300 ppm correlated with increased incidences of hepatic centriacinar fatty vacuolation. Increased thyroid weights at 30 and 300 ppm were associated with follicular epithelial hypertrophy in the F_0 and F_1 adults (except for females fed 30 ppm). This was also seen in F_1 males given 3 ppm. Absolute pituitary weights were low in F_1 females fed 30 and 300 ppm. Toxicity was observed in F_{1A} and F_2 offspring at the 300 ppm high dose level as evidenced by reduced bodyweight gain during lactation and an associated slight delay in certain developmental milestones (tooth eruption in F_{1A} litters and pinna unfolding in F_2 litters). Pre- and post-natal viability and bodyweight gain were also reduced in both the F_{1A} and F_2 offspring. Viability indices at birth and Day 4 *post partum* (prior to culling) were 83 and 89%, respectively, for the F_{1A} offspring and 78 and 73%, respectively, for the F_2 offspring compared with control values of 97 to 100%. In addition, convulsions were observed in thirteen F_1A offspring and five F_2 pups between Days 14 to 20 *post partum* when they were starting to consume the diet. No other abnormalities were evident. At 30 ppm of Fipronil there were increases in liver and thyroid weights (absolute and relative weights) of both sexes of both adult generations. Follicular epithelial hypertrophy in the thyroid, the only histopathological finding, was seen in F_0 males and both sexes of the F_1 adults. The NOAEL for systemic toxicity was 3 ppm (equivalent to 0.25 mg/kg bw/d for males or 0.27 mg/kg bw/d for females). The NOEL for reproductive and developmental toxicity was 30 ppm (equivalent to 2.54 mg/kg bw/d for males or 2.74 mg/kg bw/d for females).

Neurotoxicity:
Fipronil was evaluated in the following neurotoxicity studies in rats: two acute oral studies, a 90-day dietary study and a developmental neurotoxicity test. A 14-day repeat dose (capsular) study with a 28-day off-dose recovery period was also conducted in the dog.

Acute neurotoxicity in rats
In the first acute study, groups of Sprague-Dawley rats were given 0.5, 5.0 or 50 mg/kg bw of Fipronil in corn oil, and examined for 14 days post-treatment. Oral administration of 50 mg/kg bw, the highest dose level, resulted in death and a variety of changes in nervous system function. Slight functional neural changes were also seen at 5.0 mg/kg bw. At 50 mg/kg bw, various toxic responses were seen, including mortality and reductions in bodyweight gain and food consumption, which generally occurred within 2 days of dosing. Changes to nervous system function were also noted principally 7 hours post dosing. They included convulsions, tremors, head bobbing and myoclonic movements, decreases in hind leg splay, arousal and rearing activity in the open field, and in several reflexes (including approach response, tail pinch and air righting reflex). Reductions in muscle tone, pupil size, body temperature and motor activity as well as altered gait were also observed 7 or 8 hours post-dosing. At 7 days post dosing, there was an apparent stimulation in open-field activity in the high dose level males. The only treatment-related finding at 5 mg/kg was a decrease in hind limb splay 7 hours after dosing. All of these findings, apart from the reduction in bodyweight, were reversible and had resolved by termination, Day 14 post dosing. No treatment-related macroscopic or microscopic neural tissue findings were found. The No Observed Effect Level (NOEL) was 0.5 mg/kg bw. However,

effects at 5 mg/kg bw were limited to a decrease in hind leg splay in both sexes 7 hours after dosing. Therefore, this dose level was defined as the Lowest Observed Effect Level (LOEL).

The second acute neurotoxicity study was designed to define the No Observed Effect Level (NOEL) more precisely. Groups of Sprague-Dawley rats were given 2.5, 7.5 or 25 mg/kg bw of Fipronil in corn oil and examined for 14 days post-treatment. The highest dose level (25 mg/kg bw) reduced bodyweight gain and food consumption and impaired food efficiency during the week following treatment. It also induced a variety of functional neurological changes that were generally confined to the 7-hour post dosing time point. One male and one female showed unusual behaviour and posture. Decreases in landing hind limb splay and body temperature occurred in both sexes whilst forelimb grip strength was increased in males. Locomotor activity was also reduced during the first 10 minutes of the 7-hours post-treatment time point. At the intermediate dose level (7.5 mg/kg), bodyweight gain, food consumption and food conversion efficiency was reduced in females during the first week post dosing. Behavioural findings were limited to decreased hind leg splay in males 7 hours after dosing.
The No Observed Effect Level (NOEL) for both behavioural and general toxicity for this study was 2.5 mg/kg bw. The Lowest Observed Effect Level (LOEL) was 7.5 mg/kg bw based on a transient decrease in hind leg splay in males. Considering these two acute studies together, the clear NOEL for acute oral neurotoxicity in rats was 2.5 mg/kg bw and the LOEL was 5 mg/kg bw.

<u>90-day dietary neurotoxicity in rats</u>
In this 90-day study, groups of Sprague-Dawley rats were given continuous dietary administration of 0.5, 5.0 or 150 ppm of Fipronil. The only findings were transient reductions in bodyweight gain and food consumption at the 150 ppm highest dose level during the first one or two weeks of treatment. No evidence of neurological changes, including no histopathological effects, was seen. The No Observed Effect Level (NOEL) for neurotoxicity was 150 ppm (corresponding to 8.1 mg/kg bw/day in males and 10.8 mg/kg bw/day in females). The No Observed Effect Level (NOEL) for general toxicity was 5 ppm (corresponding to 0.3 mg/kg bw/day in males and 0.4 mg/kg bw/day in females).

<u>Conclusion on neurotoxicity</u>
Fipronil has been shown to be a potent blocker of the GABA (γ-aminobutyric acid) regulated chloride channel. At sufficient doses, Fipronil is capable of producing clinical signs of neurotoxicity, as would be expected from the compound's interaction at a neurotransmitter receptor. Clinical signs of toxicity are relatively consistent between species following single and repeated dosing. Near lethal doses are required to produce aggressive or irritable behavior, tremors, convulsions, altered gait, hunched posture, decreased motor activity, and changes in reflex responses following short term dosing while convulsions were observed at lower doses upon chronic administration to rats. No histopathological findings are observed in the nervous system after acute or short-term exposures to Fipronil. In general rats showed recovery following cessation of treatment. In the absence of neurohistopathological changes, and with no evidence of continuing behavioral abnormalities after withdrawal of treatment, the effect of Fipronil on the nervous system is concluded to be transient and pharmacological in nature.

Human data:

Medical surveillance data on manufacturing plant personnel

No human cases of intoxication deriving from fipronil in the course of production, transportation, formulation and packaging have been reported to us. Regular medical examinations are performed in accordance with a specific company-wide Fipronil Policy.

Direct observation

Mohamed et al. (J Toxicol Clin Toxicol 2004 ; 42 :955-963) reported seven prospectively documented cases of fipronil poisoning (ingestion of Regent 50 SC) in three Sri Lankan hospitals, only two had significant central nervous system toxicity. Non-sustained generalized tonic-clonic seizures were observed; both were managed without complications with benzodiazepines and supportive care. One of these two patients who ingested about 100 ml Regent 50 SC presented also with excessive sweating, uncontrollable nausea, vomiting and retching. The other pf these two presented also with nausea and vomiting. A third case had nausea, vomiting, and heart burn while the rest did not have any symptoms. Retrospective review of >1000 pesticide poisoning deaths since 1995 found only one case supposed to have ingested fipronil. In contrast to the good outcome of the above seven cases, this patient required intubation and ventilation and had continuous fits despite therapy with barbiturates and benzodiazepines, but he may have taken a much larger dose or even another unknown poison.

After ingestion of an ant bait containing about 0.14 mg fipronil only mild subjective impairment of the sensorium was reported to be experienced for half an hour (Fung et al., J Toxicol Clin Toxicol 2003; 41 :245-248).

After spraying his field with a dilute fipronil solution a 50 year old man complained of headache, nausea, vertigo, and weakness, all symptoms resolving spontaneously after 5 hours (Chodorowski and Anand, J Toxicol Clin Toxicol 2004 ; 42 :189-190)

The French Antipoison Centres reported 410 cases of human exposure to fipronil-containing insecticide formulations from 1994 to 2004. Symptoms, if present at all, were considered as mild and ascribed to solvents or adjuvants.

Dublin, Edinburgh, Göttingen, Zürich, and Perth Antipoison Centres collected information on 138 human exposures; they all (except the one described below) reported only minor symptoms such as vomiting and dizziness after ingestion and irritation after inhalation and eye contact.

Health records

Company health records do not show any fipronil-related health effects.

Diagnosis of poisoning

Fipronil is a reversible γ-aminobutyric acid (GABA) receptor inhibitor. During intoxication, it will induce neurological stimulation with possible convulsions. Most relevant signs and symptoms in humans after acute or repeated overexposure are expected to be related to central nervous system (CNS) hyperexcitability: hyperactivity, irritability, tremors and at a more severe stage lethargy or convulsions.

Due to slow absorption through the gut, symptoms of intoxication may be delayed for several hours to one day.

Measurement of fipronil and its metabolites in the blood (or in the gastric lavage) is the only way to confirm any exposure. In cases of suspected intoxication evidenced by symptoms, a blood sample should be taken as soon after the alleged exposure.

The most reliable method for determination of fipronil and its main metabolite, the sulphone, seems to be HPLC/MS.

Sensitisation/allergenicity observations

No human cases of sensitisation / allergenicity deriving from fipronil have been reported.

Specific treatment in case of accident

See safety data sheet/precautions; symptomatic and supportive treatment.

After ingestion: if possible within 60 minutes after ingestion, gastric lavage might be considered although its efficacy has not been proven.

Specific anti-convulsive therapy
Recommendations are based on anti-convulsive therapy as routinely administered to humans.

In cases of strong clinical indications of fipronil poisoning, do not wait for analytical confirmation to start treatment. Patients may present marked resistance to the usual therapeutic doses of anti-convulsive drugs. Exact dosage depends on the severity of the intoxication, the bodyweight and the reaction of the patient to the treatment.

Diazepam: Start with 10 to 30 mg diazepam by intravenous injection according to body weight. This dose is to be repeated every 10 to 30 minutes according to the patient's response.

If the patient is not responsive to the suggested treatment, or if diazepam is not available, other benzodiazepines or barbiturates can be used.

Even when symptoms of fipronil intoxication are rapidly reversed by treatment, the treatment must be continued for several days, gradually decreasing the dose of the anti-convulsive drug based on the patient's clinical response. This is necessary due to the slow elimination of fipronil. Patients who have had seizures need to be monitored until anti-convulsive treatment can be completely stopped. When the blood level of fipronil and its metabolites is lower than the convulsion threshold, no additional treatment should be needed.

Prognosis following poisoning

Fipronil is a reversible γ-aminobutyric acid (GABA) receptor inhibitor. During intoxication, it will induce neurological stimulation with possible convulsions. Most relevant signs and symptoms in humans after acute or repeated overexposure are expected to be related to central nervous system (CNS) hyperexcitability: hyperactivity, irritability, tremors and at a more severe stage lethargy or convulsions.

Due to slow absorption through the gut, symptoms of intoxication may be delayed for several hours to one day.

There are no reports of long-term sequelae or irreversible health effects available (except one death reported to be related to fipronil); they may be expected only after severe lethargy, convulsions, or coma.

3.11 Other data –Mechanistic investigations of thyroid tumourigenesis in the rat:

The results of three mechanistic studies with Fipronil on thyroid function in the rat demonstrate that the imbalance of thyroid hormone induced by Fipronil is due to a disturbance of the thyroid-pituitary hormonal feedback.

This disturbance appears to be related to an increase in the biliary clearance of T_4 rather than a direct effect on the thyroid. Uptake of radiolabelled iodine into the thyroid was significantly increased in male rats following administration of Fipronil at 10 mg/kg bw/d for 14 days. Administration of

potassium perchlorate, which causes the release of any free iodine accumulated in the thyroid, to Fipronil-treated rats resulted in no change in the levels of ^{125}I in either the thyroid or blood. These results indicate that Fipronil does not inhibit the synthesis of T_4 or T_3. Significant differences in the pharmacokinetic profile of T_4 in the blood have been observed in male rats administered ^{125}I-T_4 following oral administration of Fipronil for 14 days at 10 mg/kg bw/d. These differences consisted of a 48% decrease in the terminal half-life, a 161% increase in clearance, and a 37% increase in the volume of distribution for ^{125}I-T_4 in Fipronil-treated animals compared to controls. Thus, the decreased serum levels of T_4 observed in the long-term rat study are due to an increased clearance of this hormone probably via biliary excretion.

Normally, T_4 is secreted from the thyroid at about 8 to 10 times the rate of T_3, the physiologically more active hormone (Hill *et al*., 1989). Thus, T_4 is present in the circulation in much larger quantities than T_3, and the pituitary is effectively responding to the level of circulating T_4 (Thomas and Williams, 1992). A decrease in circulating T_4 results in an increased release of TSH from the pituitary. Under conditions of increased and sustained release of TSH, thyroid hypertrophy, hyperplasia, and ultimately tumours may be produced (Hill *et al*., 1989). Excessive secretion of endogenous TSH alone (in the absence of any chemical treatment) produces a high incidence of thyroid tumours (McClain, 1989). For example, thyroid tumours have been observed in rats fed diets deficient in iodine (Axelrad and Leblond, 1955 ; Bielschowsky, 1953 ; Isler *et al*., 1958; Leblond *et al* 1957), by subtotal thyroidectomy (Dent *et al*., 1956), and by transplantation of anterior pituitary gland tumours producing TSH (Dent *et al*., 1956; Brachetto-Brian and Grinberg, 1951; Haran-Ghera *et al*., 1960). These experimental techniques indicate that the mechanism of tumour induction resides within the animal and is dependent on an imbalance in the pituitary-thyroid axis.

With Fipronil, significant decreases in circulating T_4 levels and significant increases in circulating TSH levels were observed while T_3 levels were altered only slightly. Hypertrophy of the follicular epithelium and follicular cell hyperplasia were observed after sub-chronic (13 weeks) exposure while thyroid tumours were seen following lifetime exposure. However, a threshold level of thyroid stimulation must be achieved to initiate the sequence of events leading to tumour formation. If the hormone imbalance is insufficient to induce the pharmacological effects of thyroid hypertrophy and hyperplasia or the hormonal balance is restored and the thyroid recovers, then typically, tumours do not develop. The absence of histopathological changes in the thyroid at 30 ppm in both the subchronic and chronic rat studies despite changes in the levels of TSH and T_4 indicates that the threshold required for tumour induction was not achieved at this dietary level.

Non-genotoxic compounds that induce thyroid follicular tumours via long-term hormonal imbalance are considered to act via a threshold mechanism. For these compounds, an imbalance of thyroid hormones must occur and must be sufficient to produce a prolonged stimulation of the thyroid resulting in hypertrophy and hyperplasia and ultimately tumours. A dose level that does not cause elevation of TSH will not induce tumours in the rat thyroid. Fipronil is not genotoxic and induces thyroid tumours in the rat only at a dose level producing pharmacological effects plus thyroid hypertrophy and hyperplasia. No thyroid tumours were observed in rats at Fipronil doses which do not produce thyroid hypertrophy and hyperplasia.

The rat is known to be very sensitive to compounds affecting thyroid hormone balance. Species differences in sensitivity to an imbalance in thyroid hormones and subsequent effects on the thyroid have also been shown for other compounds such as sulfonamides and aminothiazole (Steinhoff *et al*., 1983 ; Swarm *et al*., 1973) and the Calcium-antagonist Diproteverine (Flack *et al*., 1989). For Fipronil, effects on the thyroid were observed only in rats and not in mice or dogs. These species differences may be due to differences in the half-life of thyroid hormones in rodent versus primates (12 to 24 hours in rat compared to 5 to 9 days in humans) and differences in the responsiveness of thyroid cells to TSH (Atterwill *et al.*, 1992). In view of the known sensitivity of the rat to the development of thyroid

lesions, the occurrence of thyroid lesions in the rat has no practical relevance for human risk assessment.

The opinion that Fipronil does not pose a carcinogenic hazard to humans was shared by the Meeting of the Technical Committee on Classification and Labelling of Dangerous Substances, which in 2004 decided not to propose labelling Fipronil with R40 (Limited evidence of a carcinogenic effect).

10.6 Section A7 Ecotoxicological Profile including Environmental Fate and Behaviour

Biodegradation

Fipronil attained 47% degradation after 28 days. According to OECD criteria a test material may be considered to be readily degradable if > 60% degradation is attained after 28 days. Therefore, since there was only 47% degradation, fipronil cannot be considered readily degradable under the strict terms and conditions of the OECD guidelines.

Abiotic degradation

Fipronil has been shown to be hydrolytically stable at environmentally relevant pH values. The hydrolytic stability of [^{14}C]-Fipronil, was studied in the dark, under sterile conditions, at pHs 5, 7 and 9. At pHs 5 and 7, TLC and HPLC data showed that fipronil was hydrolytically stable At pH 9 the rate of conversion is best modelled by pseudo-first order kinetics with a half life of 28 days and a rate constant k = 0.0243 day^{-1}.

The hydrolytic stability of [^{14}C]-Fipronil, was studied at pH 5 at 25 ± 1°C, under sterile conditions. Two degradation products were formed: the major organic extract photo-product was MB 46513 (43.4 % of the applied radioactivity) and a minor component (HPLC RT = 2 min) accounting for 4.0% of applied radioactivity. The kinetics of photolytic degradation were first order with a half-life of 3.63 hours under the xenon lamp corresponding to 0.33 days of summer sunlight in Florida and a rate constant k = -0.0176 days^{-1}. Photolysis can be considered a major route of fipronil degradation should it reach the aqueous environment.

A [^{14}C]-Fipronil degradation in two water/sediment systems showed that in an aerobic aquatic environment, fipronil partitions steadily into the underlying sediment where it degrades by reduction to MB 45950. MB 45950 is further degraded by hydrolysis to MB 46126. Fipronil is also hydrolysed to RPA 200766 and, to a much lesser extent oxidised too MB 46136. There is evidence than RPA 200766 and MB 46136 are further transformed to RPA 10530 via oxidation or hydrolysis respectively. The results of this study show that fipronil will not persist in an aerobic aquatic environment.

Aerobic degradation in soil

The degradation of [^{14}C]-Fipronil was investigated in two soils. The half life of [^{14}C]-Fipronil determined by HPLC in a UK sandy loam soil and a German sandy soil under aerobic conditions were 128 and 308 days respectively, degradation proceeded via hydrolysis to RPA 200766 and oxidation to MB 46136. (Waring, 1993).

In a study of [^{14}C]-Fipronil degradation in four soils (at 20°C) and two soils (at 10°C)
Fipronil was steadily degraded under aerobic conditions by hydrolysis to RPA 200766 and by oxidation to MB 46136. The rate of degradation was temperature dependent with more rapid degradation at 20°C than 10°C. The rate of degradation was also related to the soil microbial biomass activity.

The reduced metabolite MB 45950 was found in minor quantities, except in one soil where there was reduced oxygen status under these laboratory conditions. Several other minor metabolites were also observed, the hydrolysis products RP 200761 and RPA 105320 in the high pH and high biomass soil.

The DT_{50} of fipronil ranged from 26 to 296 days at 20°C and the DT_{90} from 85 to 982 days. It was not possible to derive the DT values for the metabolites.

Adsorption onto / desorption from soils

The soil adsorption/desorption properties of [^{14}C]-Fipronil were investigated using five European soil types using the slurry technique. The adsorption constants (K) obtained ranged from 4.19 in a UK sandy loam to 20.69 in a UK loam. The value of K increased with increasing organic carbon content of the soil suggesting that more fipronil was adsorbed. The K_{OC} values obtained ranged from 427 to 1248 with a mean of 727. The Freundlich desorption constants increased with the increasing desorption cycles, the results suggest that the adsorption was reversible with similar processes involved in the desorption as the adsorption. . The results indicated that fipronil is unlikely to demonstrate significant mobility in soil due to its relatively high sorption t soil. According to McCall's designation, fipronil would be expected to show medium to low mobility.

Field soil dissipation

The environmental behaviour of fipronil and its metabolites was studied following the soil incorporation of an experimental wettable powder at two location in Northern Europe.

The trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to less than half in mid application samples (6 to 9 months after application) and below or close to the limit of quantification (LOQ), when averaged over the soil profile to 30 cm, one year after application at the two sites.

The major metabolite found was MB46136 with significant amounts of RPA 200766. MB 46136 residues generally peaked at the mid-way sampling point between applications but residues were still present one year after each application. The amounts of RPA 200766 residue were less than for MB 46136 but the trend was similar. The pattern of residues for MB45950 was similar to MB 46136 but the amounts of MB 45950 found were only about 10 to 20% of MB 46136. Residues of the photolyte, MB 46513, remained below LOQ for both trials, which is consistent with soil incorporation of the parent compound.
The residues of the metabolites MB 46136, RPA 200766 and MB 45950 which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.

The environmental behaviour of fipronil and its metabolites was studied following the soil incorporation of an experimental wettable powder at two location in Southern Europe.

Based on the analytical results available up to the fifth and sixth years for the trials in France and Italy respectively, the trends in soil residues of fipronil and its metabolites were similar for the two trials. Residues of fipronil declined to below LOQ by one year after application at both trial sites. No residues of the photolyte, MB 46513, were found (apart from 2 detects) which is consistent with soil incorporation of the parent compound. The residues of the metabolites MB 46136, RPA 200766 and MB 45950 which were measured have to be put into context with mathematical predictions of the accumulation behaviour to judge on the level of the accumulation plateau that is obtained in the course of the study.

Soil mobility

In a column leaching study on five European soils fipronil was shown to have a low mobility in soil.

Bioconcentration

The bioconcentration factor and bioaccumulation potential of [14C]-labelled fipronil were measured in a fish species (bluegill, *Lepomis macrochirus*). The test comprised an uptake phase (continuous flow-

through over 35 days) and a depuration stage (14 days continuous flow-through in untreated medium). The uptake kinetics were considered to approach a simple 2-compartment model with measured BCF at steady state close to theoretical values predicted based on the log P_{ow}. The bioconcentration factor (BCF) estimated in whole fish was 321. Uptake residues were rapidly and nearly completely (>99%) eliminated from whole fish within the 14-day depuration phase. The results of this study indicate no concerns of bioaccumulation of fipronil in aquatic animals.

Effects on aquatic organisms

Acute toxicity to fish
The acute toxicity of fipronil to juvenile Bluegill sunfish (*Lepomis macrochirus*) was studied under laboratory conditions during 96 hours of flow-through exposure. The 96-h LC_{50} was calculated at 85.2 µg a.s./l. Based on the absence of lethal or sublethal effects observed up to this concentration, the 96-h NOEC was determined at 43.2 µg a.s./l.

Acute toxicity to invertebrates
The acute toxicity of fipronil to neonate *Daphnia magna* (<24 hours old) was studied in a 48 hour flow through test in the laboratory. Treatment related immobilisation and lethargy among surviving daphnids were observed at 160 and 280 µg a.s./l. The 48-h EC_{50} was calculated at 190 µg a.s./l and the NOEC was 52 µg a.s./l.

Daphnids are less sensitive to fipronil than other invertebrates, particularly insects. Therefore, testing with fipronil has been conducted on additional species of aquatic invertebrates, including insects. The acute toxicity of fipronil to the Mayfly *Hexagenia spp* was studied in a 96-h static renewal laboratory test. The 96-h LC_{50} value derived from this study was calculated to be 0.44 µg a.s./l and the NOEC was 0.14 µg a.s./l.

Growth inhibition in Algae
The algastatic activity of fipronil was measured in a 96-h laboratory study using *Scenedesmus subspicatus*. No effect on biomass production, growth rate or appearance of the alga cultures was observed at test concentrations of 10, 20 and 40 µg/l. However, effects on both biomass production and maximum growth rate as well as abnormal appearance (colour and shape) of the algae were observed at 80 and 160µg a.s./l. The E_rC_{50} and E_bC_{50} were determined to be 74 µg a.s./l and 68µg a.s./l respectively. The NOE_rC was 40 µg a.s./l.

Inhibition to microbiological activity
The effects of fipronil on the microbiological activity in an aquatic medium with a mixed inoculum of microorganisms were studied in an activated sludge respiration inhibition test under laboratory conditions. The test substance was a straight formulation of fipronil containing 800 g fipronil/kg. After 3-h exposure, the respiration rate of the activated sludged was similar in the negative control and all the fipronil concentrations (ranging from 10 to 1000 mg a.s./l). As the activity of activated sludge was not affected at the highest concentration tested, the toxicity values derived from this study are: 3-h EC_{50} > 1000 mg a.s./l and NOEC= 1000 mg a.s./l.

Effects on aquatic organisms, further studies

Chronic toxicity to an appropriate species of fish
The chronic toxicity of fipronil to rainbow trout (*Oncorhynchus mykiss*) was investigated during an early life-stage exposure in a 90-day (60-day post hatch) flow through test. The LOEC for this study was determined to be 26 µg a.s./l and the NOEC was 15 µg a.s./l.

Chronic toxicity to appropriate invertebrate species
The chronic toxicity of fipronil to neonate *Daphnia magna* (< 24 hours old) was studied in a 21-day flow through test in the laboratory. The NOEC was determined to be 9.8 µg a.s./l based on mean body length and 20 µg a.s./l based of survival and reproductive performance.

Since aquatic insects are more sensitive to fipronil than daphnids, chronic testing has also been carried out on insect species. The toxicity of ^{14}C Fipronil to the larvae of the midge species *Chironomus riparius* was investigated in a water/sediment system in the laboratory where the test substance was applied to the overlying water. This was a static test with an exposure duration of 28 days. Based on

initial measured concentrations in overlying water, the LOEC was 0.234 µg a.s./l and the NOEC was 0.117 µg a.s./l.

Effects on sediment dwelling organisms
The toxicity of sediment-applied fipronil to larvae of the midge species *Chironomus tentans* was determined in a long-term (10 days of exposure) spiked-sediment laboratory test. The 10-day LC50 was 30 µg a.s./kg and the NOEC was 16 µg a.s./kg. Although there are other long-term studies with *Chironumus* and fipronil, this result is the most relevant one because this it is the only test where the test substance was directly mixed into the sediment.

Aquatic plant toxicity
The toxicity of fipronil to the freshwater macrophyte *Lemna gibba* was studied in a 14-day static limit test in the laboratoy. There were no effects at the concentration tested. Therefore, the 14-d EC50 of fipronil in *Lemna gibba* is > 160 µg a.s./l and the 14-d NOEC is ≥ 160 µg a.s./l.

Effects on terrestrial organisms

Effects on soil micro-organisms
The effects of fipronil on the inhibition of the microbiological activity in natural soil was studied in the laboratory following exposure for 28 days. There was no adverse effects on soil respiration or nitrogen turnover at the two rates tested, which were equivalent to soil concentrations of 0.133 and 0.667 mg a.s./kg soil. Therefore, the NOEC (inhibition effects below 25%) was 0.677 mg a.s./kg soil.

Acute toxicity to earthworms
The acute toxicity of fipronil to earthworms (*Eisenia foetida*) was measured in a laboratory study conducted over 14 days in artificial soil according to the OECD Guideline No 207. The LC_{50} of fipronil was found to be greater than 1000 mg/kg. The NOEC was 1000 mg a.s./kg on the basis that no significant mortalities or adverse effects were observed after 14 days exposure.

Chronic toxicity to earthworms
The effects on reproduction and chronic toxicity of fipronil to earthworms (*Eisenia foetida*) were measured in a study conducted over 8 weeks of exposure in artificial soil. No adverse effects on survival, growth or reproduction were observed at any of the concentrations tested. The NOEC from this study was 1000 mg a.s./kg soil, the highest concentration tested.

The results of the acute and chronic tests indicate that fipronil is practically non-toxic to earthworms.

Effects on non-target arthropods – Toxicity to non-target soil-dwelling arthropods
An aged residue study was carried out to determine the duration of effects of Fipronil on the soil-dwelling arthropod beetle *Aleochara bilineata*. Soil was applied under field conditions at rates corresponding to nominal soil concentrations of 0.625, 1.25 and 2.5 mg a.s./kg soil. In total, 7 bioassays were carried out exposing *A. bilineata* to fresh and aged soil samples. One bioassay started directly after application (DAT 0) and further started 4,10,15,20,25 and 30 weeks after application (WAA). In parallel an analytical study was conducted to determine the actual concentration of fipronil and metabolites in the soil samples taken for the bioassays with *A. bilineata*. Based on the results of all bioassays and on measured concentrations of fipronil and major metabolites in soil, acceptable effects were observed at a concentration corresponding to 0.243 mg total residues/ kg soil.

Effects on birds - Acute oral toxicity
The acute oral toxicity of fipronil to bobwhite quail *(Colinius viginanus)* was measured in a 21-d LD_{50} study following exposure to single dosing. The LD_{50} of fipronil in bobwhite quail was determined at 11.3 mg ai/kg.

Effects on birds - Short term toxicity
The short-term dietary toxicity of fipronil to bobwhite quail *(Colinius viginanus)* was measured in a 22-d LC_{50} study. The LC50 was determined to be 48.0 mg a.s./kg diet and the NOEL was 19.5 mg a.s./kg diet.

Effects on birds - Effects on reproduction
The chronic toxicity of fipronil on the adults and reproduction performance of the bobwhite quail *(Colinius viginanus)* was measured in a 22-week dietary administration test in the laboratory. The study did not found direct adverse effects on reproductive paramenters but parental toxicity. Based on the overall results of the study, the NOEC was 10 mg a.s./kg diet.

Effects on terrestrial plants
The effects of fipronil (applied as a straight formulation containing 800 g fipronil/kg) on the seedling emergence of six species of plants (3 monocots and 3 dicots) were studied under greenhouse conditions. The test substance was mixed into the soil and then seeds of the six species were planted into the treated soil. The duration of exposure ranged from 21 to 25 days depending of the species. There were no adverse effects on seedling emergence and phytotoxicity at any of the soil concentrations tested (0.125, 0.5 and 2.0 mg a.s./kg soil). Plant weight at the end of exposure was not affected in four species, for which the NOEC is therefoe 2.0 mg a.s./kg. In two species (oilseed rape and oats) there was a reduction in plant weight at the highest concentration. Based on the results of these two species, the overall NOEC of the study is 0.5 mg a.s./kg soil.

Effects on Honeybees - Acute toxicity
The acute oral and contact toxicity of fipronil to honey bees (*Apis mellifera*) was studies in the laboratory. The acute oral and contact LD50 determined were 0.00417 and 0.00593 µg a.s./bee, respectively.

10.7 Section A8 Measures Necessary to Protect Man, Animals and the Environment

8.1	**Recommended methods and precautions concerning handling, use, storage, transport or fire:**	
	Precautions to be taken in handling and storing:	**Handling :** When using do not eat, drink or smoke **Storage :** Keep out of the reach of children Keep away from food, drink and animal feedingstuffs Keep out of the light
	Main Hazards	**Human health hazards:** Toxic by inhalation, contact with the skin an by ingestion Danger of serious damage to health by prolonged exposure if swallowed **Environmental hazards:** Very toxic to aquatic organisms May cause long-term adverse effects in the aquatic environment

Exposure control/ Personal protection:	**Engineering measures** Keep the product as confined as possible. Capture dust at the point of emission **Hygiene measures** Keep the work place and equipment clean **Personal protective equipment** Filter dust mask, ventilated hood or ventilated coverall depending on exposure Wear gloves, coveralls and glasses		
Transport:	**Transport Label; Solid pesticide, toxic**		
	RID/ADR Classe: 6.1	UNN°: 2588	Label 6.1
	MARITIME Classe: 6.1	UNN°: 2588 Page IMDG:6221	Marine Pollutant : Yes Label 6.1
	AIR Classe: 6.1	UNN°: 2588 Labelling group: III	Label 6.1
Flash Point:	Not applicable		
Extinguishing Media:	Water, foam, carbon dioxide or powder		
Special Fire fighting Procedures:	Limit the spread of extinguishing media Wear suitable respiratory equipment		
Usual Fire and Explosion Hazards:	Combustible, risk of toxic gasses in the fumes		
Reactivity Data:	No dangerous reactions known under the normal conditions of use		
Stability:	Stable under normal conditions		
Conditions to avoid:	None		
Incompatibility (Materials to Avoid):	None		
Hazardous Decomposition Products:	None		
Hazardous Polymerization:	None		
Conditions to avoid:	None		

	Steps to be taken in case material is released or spilled:	**Personal protection:** Filter dust mask, ventilated hood or ventilated coverall depending on exposure. Wear gloves, coveralls and glasses. **Environmental precautions:** Use appropriate containment to avid environmental contamination. **Spillages:** Recover the product by dampening and sweeping up or by vacuum. The material and its container must be disposed of as hazardous waste.
	Other Precautions:	None
	Regulatory Information:	**Labelling:** **R phrases:** 23/24/25-48/25-50/53 **S phrases:** (1/2-)-13-20/21-36/37-45-60-61
8.2	**Specific treatment in case of an accident e.g. first aid measures, antidotes, medical treatment if available; emergency measures to protect the environment**	
	First Aid Measures:	**Inhalation**: Keep patient calm, remove to fresh air, seek medical attention. **Ingestion:** Rinse mouth immediately and then drink plenty of water, seek medical attention. Do not induce vomiting unless told by a poison control center or doctor. Never induce vomiting or give anything by mouth if the victim is unconscious or having convulsions. **Skin contact:** Immediately wash thoroughly with soap and water, seek medical attention. **Eye contact:** Immediately wash affected eyes for at least 15 minutes under running water with eyelids held open, consult an eye specialist.
	Emergency measures to protect the environment:	Recover the product by dampening and sweeping up or by vacuum. Use appropriate containment to avid environmental contamination. The material and its container must be disposed of as hazardous waste.
8.3	**Procedures, if any for cleaning application equipment**	
		Not applicable
8.4	**Identity of relevant combustion products in cases of fire:**	
		Oxides of carbon, sulphur and nitrogen, hydrochloric and hydrofluoric acid.
8.5	**Procedures for waste management of the biocidal product and its packaging for industry, professional users and the general public (non-professional users), e.g.: possibility of re-use or recycling, neutralisation, conditions for controlled discharge and incineration**	

	Product disposal:	Dispose of this material and its container at hazardous or special waste collection point. Incinerate at a specialist facility – minimum temperature 1150°C minimum residence time 2 seconds.
	Container disposal:	Commercial containers must be completely empty. As for product/container.
8.6	**Possibilities of destruction or decontamination following release in or on the following:** **(a) Air** **(b) Water, including drinking water** **(c) Soil**	
8.7	**Observations on undesirable or unintended side-effects, e.g. on beneficial or other non-target organisms**	
		Very toxic to aquatic organisms, toxic to bees, Dangerous for terrestrial fauna.
8.8	**Specify any repellents or poison control measures included in the preparation that are present to prevent action against non-target organisms.**	
		none

10.8 Section A9 Classification and Labelling

10.8.1 Current classification

Table A10.8.1-1: Current classification of a.s. Current classification according to Annex I of Council Directive 67/548/EEC

Classification	T N	Toxic Dangerous to the Environment
Class of danger	Toxic by inhalation, in contact with the skin and by ingestion Very toxic to aquatic organisms Toxic to fauna and bees	
R phrases	23/24/25 48/25 50/53 55 57	Toxic by inhalation, in contact with skin and if swallowed Toxic: danger of serious damage to health by prolonged exposure in contact with skin Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment Toxic to fauna Toxic to bees

S phrases	45	In case of accident or if you feel unwell seek medical advice immediately (show the label where possible)
	60	This material and its container must be disposed of as hazardous waste
	61	Avoid release to the environment. Refer to special instructions/safety data sheet

Proposed classification

The above classification is accepted and is compliant with Council Directive 67/548/EEC as amended on the classification packaging and labelling of dangerous substances.

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	June 2007
Materials and methods	
Conclusion	Modifications of summary are revised by RMS in document IIA. Please refer to Document IIA for final version.
RELIABILITY	
ACCEPTABILITY	
REMARKS	
	COMMENTS FROM ...
DATE	
RESULTS AND DISCUSSION	
CONCLUSION	
RELIABILITY	
ACCEPTABILITY	
REMARKS	

Reference list by section point

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A2.10.2.2/01	Mason P	2006	Environmental risk assessment for Goliath® Gel Unpublished (XXXX)	Y	BASF
A3.1.1/01	Daum A.	2004	Determination of the melting point of fipronil (BAS 350I) (unpublished) (XXXX)	Y	BASF
A3.1.1/02	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.1.1/03	Chabassol Y.C Hunt G.M	1991	M&B46030 Physical properties GLP (unpublished) (XXXX)	Y	BASF
A3.1.2/01	Daum A.	2004	Determination of the melting point of fipronil (BAS 350I) Reg No 4020907) PAI GLP (unpublished) (XXXX)	Y	BASF
A3.1.2/02	Chabert M.S. Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.1.2/03	Chabert M.S. Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.1.3/01	Nobuhiro K	2001	Measurement of density of fipronil GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A3.2/01	Nobuhiro K	2001	Measurement of vapour pressure of fipronil (translation of the original report) GLP (unpublished) (XXXX)	Y	BASF
A3.2/02	Chabassol Y Reynaud R	1991	M&B 46030 Technical Grade Vapour Pressure Curve GLP (unpublished) (XXXX)	Y	BASF
A3.2.1/01	Bascou J P	2002	Fipronil: Henry's Law Constant Calculation GLP (Not applicable – calculation) (unpublished) (XXXX)	Y	BASF
A3.2.1/02	Chabassol Y	1992	Fipronil Henry-Constant GLP (Not applicable – calculation) (unpublished) (XXXX)	Y	BASF
A3.3.1/01	Chabassol Y Hunt G. M	1991	M&B46030 Physical properties GLP (unpublished) (XXXX)	Y	BASF
A3.3.2/01	Chabassol Y Hunt G. M	1991	M&B46030 Physical properties GLP (unpublished) (XXXX)	Y	BASF
A3.3.3/01	Chabassol Y Hunt G. M	1991	M&B46030 Physical properties GLP (unpublished) (XXXX)	Y	BASF
A3.4/01	Muehlberger B	2001	AE F124964/MB046030 Spectral Data (UV/VIS) and Molar Extinction Coefficient GLP (unpublished) (XXXX)	Y	BASF
A3.4/03	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A3.4/04	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.4/05	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.4/06	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.4/07	Chabert M.S, Lecourt N.C	1996	Suitablility for use as an analytical standard reference material GLP (unpublished) (XXXX)	Y	BASF
A3.5/01	Daum A.	2005	Determination of the water solubility of fipronil(BAS 350I) Reg No 4020907) PAI GLP (unpublished) (XXXX)	Y	BASF
A3.5/02	Nobuhiro K	2001	Measurement of water solubility of fipronil GLP (unpublished) (XXXX)	Y	BASF
A3.5/03	Chabassol Y.C Reynaud R	1991	M&B46030 Technical Grade: Water solubility at 20°C GLP (unpublished) (XXXX)	Y	BASF
A3.6/01	Cichy M	2001	Statement on the Dissociation Constant Aventis Crop Science GmbH GLP (Not applicable – Statement) (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A3.7/01	Chabassol Y.C Reynaud Rx	1991	M&B46030 Technical Grade: Solubility in Organic Solvents GLP (unpublished) (XXXX)	Y	BASF
A3.9/01	Chabassol Y Reynaud R	1991	M&B46030 Technical Grade Octanol/Water Partition Coefficient at 20°C GLP (unpublished) (XXXX)	Y	BASF
A3.9/02	Cousin J	1997	Fipronil Active Ingredient: n-Octanol/Water Partition Coefficient GLP (unpublished) (XXXX)	Y	BASF
A3.10/01	Daum A.	2004	Determination of the melting point of fipronil (BAS 350I) Reg No 4020907) PAI GLP (unpublished) (XXXX)	Y	BASF
A3.11/01	Cousin J Fillion J	1996	Determination of flamability and ability of self-heating of fipronil technicque GLP (unpublished) (XXXX)	Y	BASF
A3.11/02	Cousin J Fillion J	1996	Determination of flamability and ability of self-heating of fipronil technicque GLP (unpublished) (XXXX)	Y	BASF
A3.13/01	Cousin J	1996	Fipronil: Surface Tension and Particle Size Distribution Rhône-Poulenc Secteur Agro, Study No 96-125 GLP (unpublished) (XXXX)	Y	BASF
A3.15/01	Vandermarliere P	1992	Fipronil (M&B 46030) Minimum Ignition Energy, Lower Explosive Limit (Dust cloud) and Auto-ignition (layer) Non GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A3.15/02	Tran Thanh Phong J	1999	Fipronil: Explosion and Oxidising Properties GLP (unpublished) (XXXX)	Y	BASF
A3.16/01	Tran Thanh Phong J	1999	Fipronil: Explosion and Oxidising Properties GLP (unpublished) (XXXX)	Y	BASF
A3.17/01	Cousin, J	1997	Wet technical fipronil; storage stability GLP (unpublished) (XXXX)	Y	BASF
A4.1/01	Robles J.M, Cousin J	1996	Technical Fipronil HPLC determination of active ingredient. 96 GLP (unpublished) (XXXX)	Y	BASF
A4.2.1/01	Ballesteros, C. Claviere, B. Kieken, J-L	2000	Fipronil and its metabolites (XXXX): Analytical method for the determination of residues in soil. Aventis (unpublished) (XXXX)	Y	BASF
A4.2.1/02	Grote C.	2005a	Validation of analytical method No. 547/0 - LC-MS/MS determination of BAS 350 I (Fipronil) and its metabolites XXXX in soil. GLP (unpublished) (XXXX)	Y	BASF
A4.2.3.1/01	Diot R., Kieken J.-L	2002	Validation of the method AR 163-98 for the determination of residues of Fipronil and its metabolites (XXXX) in drinking water at 0.05 µg/L (unpublished) (XXXX)	Y	BASF
A4.2.3.1/02	Bourgade C. Jendrzejczak N. Yslan, F.	1998	Fipronil and its metabolites (XXXX); Analytical method for the determination of residues in drinking water GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A4.2.3.2/01	Ibrahim A.S	1999	Validation of the method of analysis for possible residues of fipronil and its metabolites XXXX in water GLP (unpublished) (XXXX)	Y	BASF
A4.2.3.2/02	Fuchsbichler G	1999	Method validation study for Fipronil and its metabolites (XXXX) in Surface water (river, Pond) GLP (unpublished) (XXXX)	Y	BASF
A4.2.3.2/03	Lopes A.	1997	Validation of method of analysis for the determination of Fipronil and its metabolites in water GLP (unpublished) (XXXX)	Y	BASF
A4.2.3.2/04	Grote C	2005b	Validation of analytical method No. 572/0: LC-MS/MS determination of BAS 350 I (Fipronil) and its metabolites XXXX in drinking and surface water GLP (unpublished) (XXXX)	Y	BASF
A4.2.3.2/05	Grote C	2006	Validation of analytical method No. 559/0 - LC-MS/MS determination of BAS 350 I (Fipronil) and its metabolites XXXX in drinking and surface water GLP (unpublished) (XXXX)	Y	BASF
A4.2.4/01	Beaudonnet JP	1998	Fiproles determination in blood plasmas (unpublished) (XXXX)	Y	BASF
A4.2.4/02	Communal P.Y.	1994	Validation of the assay method (AGR/MOA/FIP12) of Fipronil and its metabolites (XXXX) in human plasma samples GLP (unpublished) XXXX	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A4.2.4/03	Pontal P.G.	1995	Validation of the method for the assay of Fipronil and its metabolites (MB 46136 and MB 46513) in human plasma Rhone-Poulenc Agro, Sophia Antipolis, France; Study code RPS/FIP/94111, 44 pages GLP 28 June 1995 (unpublished) XXXX	Y	BASF
A4.2.4/04	Oullier J.P & Soun A.	1995	XXXX and Metabolites (XXXX), Analytical determination method of plasma levels in micro-pigs XXXX GLP (unpublished) XXXX	Y	BASF
A4.2.4/05	Goller G	1998	Stability study of fipronil and its metabolites (XXXX) at -20°C and -80°C during a period of 12 months XXXX Rhone-Poulenc Agro Study code: 96-121 GLP (unpublished) XXXX		
A4.2.4/06	Bross M.	2009	Fipronil (BAS 350 I): Analytical method for body fluids Unpublished (XXXX)	Y	BASF
A4.3.1/01	Fuchsbichler G	2001	Independent laboratory validation of method study No. 98-153 for the determination of Fipronil and its metabolites XXXX in plants. GLP (unpublished) (XXXX)	Y	BASF
A4.3.2/01	Hausmann S.	1999	Multi-residue enforcement method (DFG S19) for the determination of Fipronil and its metabolites (XXXX) in foodstuff of animal origin GLP (unpublished) (XXXX)	Y	BASF
A4.3.2/02	Kerl W & Hopf B	2007	Validation of the analytical method 568/0: Method for the Determination of Fipronil and its Metabolites in Animal Matrices GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A4.3.2/03	Class T	2005	Independent Laboratory Validation of BASF Method No. 568/0 for the Determination of Fipronil and Its Metabolite XXXX in Animal Fat GLP (unpublished) (XXXX)	Y	BASF
A5.7.1/01 and	Anonymous	2006	Section 5.7: Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies	Y	BASF
A5.7.2/01 and	Anonymous	2006	Section 5.7: Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies	Y	BASF
A5.9/01	Kaakeh W, Reid B L Bennett G W	1997	The toxicity of fipronil to German and American Cockroaches (XXXX)	N	
A6.1.1/01	XXXX	XXXX	Acute oral toxicity to rats of M&B 46,030. GLP (unpublished) (XXXX)	Y	BASF
A6.1.2/01	XXXX	XXXX	Acute dermal toxicity to rats of M&B 46,030. GLP (unpublished) (XXXX)	Y	BASF
A6.1.2/02	XXXX	XXXX	M&B 46,030: Acute percutaneous toxicity study in the rabbit. GLP (unpublished) (XXXX)	Y	BASF
A6.1.3/01	XXXX	XXXX	M&B 46030: Acute inhalation toxicity study in the rat. XXXX GLP (unpublished) (XXXX)	Y	BASF
A6.1.3/02	XXXX	XXXX	Fipronil: Acute nose-only dust inhalation toxicity study in rats. XXXX (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A6.1.4/01	XXXX	XXXX	MB 46030 (technical): cutaneous irritancy study in the rabbit. XXXX GLP (unpublished) (XXXX)	Y	BASF
A6.1.4/02	XXXX	XXXX	MB 46030 (technical): Ocular irritancy study in the rabbit XXXX (unpublished) (XXXX)	Y	BASF
A6.1.5/01	XXXX	XXXX	M&B 46030: Delayed contact hypersensitivity study in Guinea-pigs. XXXX GLP (unpublished) (XXXX)	Y	BASF
A6.1.5/02	XXXX	XXXX	M&B 46030: Dermal sensitization study in guinea-pigs. GLP (unpublished) (XXXX)	Y	BASF
A6.1.5/03	XXXX	2007	Fipronil: Toxicological assessment according to the Biocidal Product Directive 98/8/EC of The EU-Dossier by the RMS France, Position paper on open points raised by the RMS XXXX (unpublished)	Y	BASF
A.6.2/01	XXXX	XXXX	(^{14}-C) M&B 46030: Absorption, distribution, metabolism and excretion in the rat. (unpublished) (XXXX)	Y	BASF
A.6.2/02	XXXX	XXXX	Addendum to Report (^{14}-C) M&B 46030: Absorption, distribution, metabolism and excretion in the rat. (XXXX)	Y	BASF
A.6.2/03	XXXX	XXXX	Fipronil: Bile Excretion Study in the rat GLP (Unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A.6.2/04	XXXX	XXXX	Dermal absorption of ^{14}C-Fipronil Regent 80 WDG in male rats (Preliminary and definitive phases). (unpublished) (XXXX)	Y	BASF
A.6.2/05	XXXX	XXXX	Fipronil: In vitro absorption from a 25 g/l ULV formulation through human and rat epidermis GLP (unpublished) (XXXX)	Y	BASF
A.6.2/06	XXXX	XXXX	Fipronil: In vitro absorption from 300 g/l EC formulation through human and rat epidermis GLP (unpublished) (XXXX)	Y	BASF
A.6.2/07	XXXX	XXXX	Fipronil: In vitro absorption from a 50 g/l SC formulation through human and rat epidermis GLP (unpublished) (XXXX)	Y	BASF
A.6.2/08	XXXX	XXXX	In vitro skin permeability of M&B 46030. GLP (unpublished) (XXXX)	Y	BASF
A.6.2/09	XXXX	XXXX	Fipronil: Tissue Kinetic study in the rat. GLP (unpublished) (XXXX)	Y	BASF
A6.3.1/01	XXXX	XXXX	M&B 46,030 Toxicity to rats by dietary administration for 4 weeks. GLP (unpublished) (XXXX)	Y	BASF
A6.3.2/01	XXXX	XXXX	M&B 46030: Twenty-one day repeated cutaneous dose toxicity study in New Zealand White rabbits #2. GLP (unpublished) (XXXX)	Y	BASF

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A6.4.1/01	XXXX	XXXX	M&B 46030: Toxicity study by dietary administration to CD rats for 13 weeks. GLP (unpublished) (XXXX)	Y	BASF
A6.4.1/02	XXXX	XXXX	M&B 46030: Toxicity by oral (capsule) administration to Beagle dogs for 13 weeks. GLP (unpublished) (XXXX)	Y	BASF
A6.4.1/03	XXXX	XXXX	M&B 46030: Toxicity by oral (capsule) administration to Beagle dogs for 52 weeks. GLP (unpublished) (XXXX)	Y	BASF
A6.4.1/04	XXXX	XXXX	M&B 46030: Toxicity study by dietary administration to Beagle dogs for 52 weeks. GLP (unpublished) (XXXX)	Y	BASF
A6.5/01	XXXX	XXXX	M&B 46030: Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks including a 13 week reversibility period on completion of 52 weeks of treatment. Final Report. GLP (unpublished) (XXXX)	Y	BASF
A6.6.1/01	XXXX	198)	Study to determine the ability of M&B 46030 to induce mutation in four histidine-requiring strains of *Salmonella typhimiurim*. GLP (unpublished) (XXXX)	Y	BASF
A6.6.1/02	XXXX	2005	Escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 350 I (Fipronil) GLP (unpublished) (XXXX)	Y	BASF

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A6.6.2/01	XXXX	XXXX	Study to evaluate the chromosome damaging potential of M&B 46030 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay. GLP (unpublished) (XXXX)	Y	BASF
A6.6.2/02	XXXX	XXXX	Fipronil: Chromosome aberration test in CHL cells *in vitro*. GLP (unpublished) (XXXX)	Y	BASF
A6.6.3/01	XXXX	XXXX	M&B 46030: Investigation of mutagenic activity at the HGPRT locus in a Chinese hamster V79 cell mutation system - Amended final report. GLP (unpublished) (XXXX)	Y	BASF
A6.6.4/01	XXXX	XXXX	M&B 46030: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test. Amended final report. GLP (unpublished) (XXXX)	Y	BASF
A6.6.4/02	XXXX	XXXX	M&B 46030: Mouse micronucleus test to comply with OECD Guideline 474 (1983) GLP (unpublished) (XXXX)	Y	BASF
A6.6.5/01	XXXX	XXXX	In vivo unscheduled DNA synthesis (UDS) assay with BAS 350 I (Fipronil) in rat hepatocytes - single oral administration. GLP (unpublished) (XXXX)	Y	BASF
A.6.6.5/02	XXXX	XXXX	Amendment No. 1 to the study report: In vivo unscheduled DNA synthesis (UDS) assay with BAS 350 I (Fipronil) in rat hepatocytes - single oral administration. GLP (unpublished) (XXXX)	Y	BASF

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A6.7/01	XXXX	XXXX	M&B 46030: Combined oncogenicity and toxicity study by dietary administration to CD rats for 104 weeks including a 13 week reversibility period on completion of 52 weeks of treatment. Final Report. GLP (unpublished) (XXXX)	Y	BASF
A6.7/02	XXXX	XXXX	M&B 46030: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. Final Report. GLP (unpublished) (XXXX)	Y	BASF
A6.8.1/01	XXXX	XXXX	The effect of M&B 46030 on pregnancy of the rat. GLP (unpublished) (XXXX)	Y	BASF
A6.8.1/02	XXXX	XXXX	M&B 46030: Teratology study in the rabbit. Final Report. GLP (unpublished) (XXXX)	Y	BASF
A6.8.2/01	XXXX	XXXX	M&B 46030: Reproductive performance study in rats treated continuously through two successive generations. Final report. GLP (unpublished) (XXXX)	Y	BASF
A6.8.2/02	XXXX	XXXX	M&B 46030: Reproductive performance study in rats treated continuously through two successive generations. Amendment to final report. XXXX GLP (unpublished) (XXXX)	Y	BASF
A6.9/01	XXXX	XXXX	M&B 46030: Single Exposure Peroral (Gavage) Neurotoxicity Study in Sprague Dawley Rats. GLP (unpublished) (XXXX)	Y	BASF

Annex point(s)	Author(s)	Date Year / Month / Day	Title Source BASF DocID GLP or GEP status Published or not	Data Protection Y/N	Owner
A6.9/02	XXXX	XXXX	Fipronil: Neurotoxicity to rats by acute oral administration (including a time to peak effect study). GLP (unpublished) (XXXX)	Y	BASF
A6.9/03	XXXX	XXXX	M&B 46030: Ninety-day dietary neurotoxicity study in Sprague Dawley rats. GLP (unpublished) (XXXX)	Y	BASF
A6.10/01	XXXX	XXXX	M+B 46,030: An investigation into the potential effects on thyroid function in male rats by studying thyroxine clearance. (unpublished) (XXXX)	Y	BASF
A6.10/02	XXXX	XXXX	M&B 46,030: An investigation into the potential effects on thyroid function in male rats using the "Perchlorate Discharge Test". GLP (unpublished) (XXXX)	Y	BASF
A6.10/03	XXXX	XXXX	The Effect of singe and repeated oral doses of M&B 46030 on the biliary excretion of intravenously administered ^{125}I-Thyroxine from bile duct cannulated rats GLP (unpublished) (XXXX)	Y	BASF
A.7.1.1.1.1/01	Corgier, M.C. and Plewa, A.P.	1992	[^{14}C] –MB46030, Hydrolysis at 25°C. GLP (unpublished) (XXXX)	Y	BASF
A.7.1.1.1.2/01	Corgier MMC; Plewa, A.P	1992	^{14}C –MB46030, Aqueous photolysis. GLP (unpublished) (XXXX)	Y	BASF
A7.1.1.2.1/01	Mead, C.	1997	Assessment of Ready Biodegradability: CO_2 Evolution Test . GLP (unpublished) (XXXX)	Y	BASF

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A.7.1.2.2.2/01	Roohi, A; Buntain I	2002.	[14C]-Fipronil: Degradation in Two Water/Sediment Systems GLP (unpublished) (XXXX)	Y	BASF
A7.1.2.2.2/02	Feung C.S., Yenne S.P.	1997	Fipronil: Aerobic aquatic metabolism GLP Unpublished XXXX 27 March 1997.	Y	BASF
A7.1.2.2.2/03	Ayliffe J.M.	1998	[14C]-Fipronil degradation and retention in two water/sediment systems GLP XXXX February 1998. (unpublished)	Y	BASF
A.7.1.3/01	Godward P.J., Austin D.J., Quarmby D.L.	1992	MB46030-14C Adsorption/desorption on five soils GLP (unpublished) (XXXX)	Y	BASF
A.7.2.1/01	Waring, A. R	1993	[14C]-M&B 46030 : Aerobic Soil Metabolism GLP (unpublished) (XXXX)	Y	BASF
A7.2.1/02	Gottesburen B.	2010	Thermograph records to address from RMS on the incubation temperature on the study Waring (1993) 13 January 2010 Unpublished XXXX	Y	BASF
A7.2.1/03	Gottesburen B.	2010	Thermograph records to address from RMS on the incubation temperature on the study Waring (1993) 09 April 2010 Unpublished XXXX	Y	BASF
A.7.2.2.1/01	Fitzmaurice, MJ. Mackenzie, E	2002	[14C]-Fipronil: Degradation in Four Soils at 20°C and Two Soils at 10°C GLP (unpulished) (XXXX)	Y	BASF

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A.7.2.2.2/01	Wicks R.	2005	Fipronil: Long term soil dissipation study in Northern Europe with repeated applications (final data after 6 years) (unpublished) (XXXX)	Y	BASF
A.7.2.2.2/02	Wicks R.	2005	Fipronil: Long term soil dissipation study in Southern Europe with repeated applications (final data after 6 years) (unpublished) (XXXX)	Y	BASF
A7.2.3.2/01	Godward, PJ; Quarmby, DL; Austin, D. J	1993.	M&B 46030^{14}C Leaching study with five soils (unpublished) (XXXX)	Y	BASF
A.7.3.1/01	Van der Gaauw, A	2001	Estimation of the degradation of Fipronil by photo-oxidation in air (unpublished) (XXXX)	Y	BASF
A.7.4.1.1/01	XXXX	XXXX.	Acute Toxicity to Bluegill, *Lepomis macrochirus*, under Flow-Through Test Conditions Toxicon Environmental Sciences USA, Study number : J9005012b GLP (unpublished) (XXXX)	Y	BASF
A.7.4.1.2/01	XXXX	XXXX.	M&B 46030 -Acute toxicity to daphnids (*Daphnia magna*) during a 48-hour flow through exposure. GLP (unpublished) (XXXX)	Y	BASF
A.7.4.1.2/02	XXXX	XXXX	Fipronil – Acute Toxicity to Mayfly Nymphs (*Hexagenia sp.)*under Static-Renewal Conditions XXXX GLP (unpublished) (XXXX)	Y	BASF
A.7.4.1.3/01	XXXX	1991	The algistatic activity of M&B 46030. GLP (XXXX)	Y	BASF

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A.7.4.1.4/01	XXXX	2001	Toxicity of EXP60720A to Activated Sludge in a Respiration Inhibition Test. GLP(unpublished) (XXXX)	Y	BASF
A.7.4.2/01	XXXX	XXXX	[^{14}C}-M&B 46030: Bioaccumulation Test in Bluegill GLP (unpublished) (XXXX)	Y	BASF
A.7.4.3.2/01	XXXX	XXXX	(M&B 46030) – The toxicity to rainbow trout (*Oncorhynchus mykiss*) during an early life-stage exposure) GLP (unpublished) (XXXX)	Y	BASF
A.7.4.3.4/01	XXXX	1995	The Chronic Toxicity of M&B 46030 to *Daphnia magna* under Flow-Through Conditions GLP (unpublished) (XXXX)	Y	BASF
A.7.4.3.4./02	XXXX	2004	Effect of ^{14}C Fipronil on the Development of Sediment Dwelling Larvae of *Chironomus riparius* in a Water Sediment System (unpublished) (XXXX)	Y	BASF
A7.4.3.4/03	XXXX	1995	**NON KEY STUDY** Fipronil - Chronic toxicity to mysids (*Mysidopsis bahia*) under flow-through conditions. (unpublished) GLP (XXXX)	Y	BASF
A7.4.3.4/04	XXXX	2005	**NON KEY STUDY** Fipronil – Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*) Under Static Conditions in a Water-Sediment System. (unpublished) GLP (XXXX)	Y	BASF

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A.7.4.3.5.1/01	XXXX	2003	Fipronil - Toxicity to midge (*Chironomus tentans*) during a 10-day sediment exposure. GLP (unpublished) (XXXX)	Y	BASF
A.7.4.3.5.1/02	XXXX	2009	Chronic toxicity of BAS 350 I (Fipronil) to the non-biting midge Chironomus riparius - a spiked sediment study.. GLP (unpublished) (XXXX)	Y	BASF
A.7.4.3.5.2/01	XXXX	1991	MB 46030 Toxicity to Duckweed, *Lemna gibba* GLP (unpublished) (XXXX)	Y	BASF
A.7.5.1.1/01	XXXX	2002	Effects of MB046030 on the activity of the soil microflora in the laboratory (AE F124964) GLP (unpublished) (XXXX)	Y	BASF
A.7.5.1.2/01	XXXX	XXXX	The Acute Toxicity of M&B46030 to Earthworms (Eisenia foetida) GLP (unpublished) (XXXX)	Y	BASF
A.7.5.1.3/01	XXXX	2004	Test to determine the effects of BAS 350 00 I on seedling emergence of terrestrial plants GLP (unpublished) (XXXX)	Y	BASF
A.7.5.1.3/02	XXXX	2005	Final report amendment No. 01: Test to determine the effects of BAS 350 00 I on seedling emergence of terrestrial plants GLP (unpublished) (XXXX)	Y	BASF
A.7.5.2.1/01	XXXX	XXXX	Effects on reproduction and growth of earthworms (*Eisenia andrei*) in artificial soil GLP (unpublished) (XXXX)	Y	BASF

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A.7.5.3.1.1/01	XXXX	XXXX	M&B 46030 technical: 21day acute oral LD50 study in bobwhite quail (*Colinius viginanus*) GLP (unpublished) (XXXX)	Y	BASF
A.7.5.3.1.2/01	XXXX	XXXX	M&B46030 technical: 22 day Acute Dietary LC_{50} Study in Bobwhite Quail GLP (unpublished) (XXXX)	Y	BASF
A.7.5.3.1.3/01	XXXX	XXXX	M&B46030 technical: Toxicity and reproduction study in bobwhite quail GLP (unpublished) (XXXX)	Y	BASF
A.7.5.4.1/01	XXXX	XXXX	The acute oral and contact toxicity of M&B 46030 to honey bees. 8 October 1991 GLP (unpublished) (XXXX)	Y	BASF
A.7.5.4.1/02	XXXX	2005	An Aged Residue Field Trial on the Effects of BAS 350 00 I on the Reproduction of Rove Beetles Aleochara bilineata, GLP (unpublished) (XXXX)	Y	BASF
A7.5.4.1/03	XXXX	2001	**NON KEY STUDY** Effects of EXP60720A on the reproduction of Rove Beetles *Aleochara bilineata* Gyll. (Coleoptera, Staphylinidae) in the Laboratory. GLP Unpublished (XXXX	Y	BASF
A7.5.4.1/04	Richter T,	2005	Determination of BAS 350 I (Fipronil) and its metabolites XXXX, XXXX, XXXX and XXXX in soil samples GLP Unpublished (XXXX) and Report Amendment (XXXX)	Y	BASF