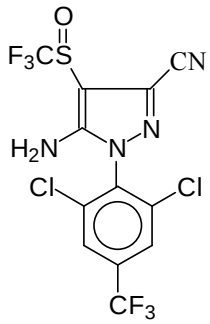
Section A1 **Annex Point IIA, I**	**Applicant**

1.1	**Applicant**	Name: BASF Agro B.V Wädenswil Branch Address: Steinacherstrasse 101 CH-8820 Wädenswil (Switzerland) Representative for BASF in Europe XXXX	
1.2	**Manufacturer of active substance**	Name: BASF Agro B.V Wädenswil Branch Address: Steinacherstrasse 101 CH-8820 Wädenswil (Switzerland) XXXX Location of manufacturing plant: XXXX	

Section A2 **Annex Point IIA, II**	**Identity of Active Substance**

Subsection (Annex Point)			**Official use only**
2.1	**Common name (IIA2.1)**	Fipronil	
2.2	**Chemical name (IIA2.2)**	IUPAC: (±)-5-amino-1-(2,6-dichloro-α,αα,-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile CAS: 5-amino-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile	X
2.3	**Manufacturer's development code number(s) (IIA2.3)**	M&B 46030 MB 46030 AE F124964 BAS 350 I	
2.4	**CAS No and EC numbers (IIA2.4)**		
2.4.1	**CAS-No**	120068-37-3	
	Isomer 1	Not relevant	
	Isomer n	Not relevant	
2.4.2	**EC-No**	424 610 5	X
	Isomer 1	Not relevant	
	Isomer n	Not relevant	
2.4.3	**Other**	CIPAC 581	
2.5	**Molecular and structural formula, molecular mass (IIA2.5)**		
2.5.1	**Molecular formula**	$C_{12}H_4Cl_2F_6N_4OS$	
2.5.2	**Structural formula**		
2.5.3	**Molecular mass**	437.15 g/mol	

Section A2 **Annex Point IIA, II**	**Identity of Active Substance**

2.6	**Method of manufacture of the active substance (IIA2.1)**	See the Business Confidential Information document IIIA-2.6 BCI.	
2.7	**Specification of the purity of the active substance, as appropriate (IIA2.7)**	g/kg 950 For range of concentration please refer to the Business Confidential Information document IIIA-2.7 BCI.	g/l % w/w 95 % v/v
2.8	**Identity of impurities and additives, as appropriate (IIA2.8)**	See the Business Confidential Information document IIIA-2.8 BCI.	
2.8.1	**Isomeric composition**	Not relevant	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA2.9)**	Not relevant	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**2.2 Chemical name** ~~*IUPAC: (±)-5-amino-1-(2,6-dichloro-α,αα,-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile*~~ IUPAC: (±)-5-amino-1-(2,6-dichloro-α,α,α,-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile ~~*CAS: 5-amino-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile*~~ *CAS:* 1H-Pyrazole-3-carbonitrile, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4[(trifluoromethyl)sulfinyl]- (+/-) is the identification for a racemat, information about the ratio of the two enantiomeric forms from the applicant: Sulfoxides can form stable R/S isomers, but in the reaction sequence there is no chiral information (raw material, solvents etc.) giving one stereoisomer priority in the synthetic pathway (racemic synthesis) over the other. Therefore a 1:1 R/S isomeric ratio is most likely. Even if a conversion of R into S and S into R might be possible, there is no chiral information within the molecule supporting the formation of one isomer. Therefore it is most likely the 1:1 R/S isomeric ratio will remain constant in the technical material. **2.4.2 EC-No** ~~*424-610-5*~~ 424-610-5
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section 2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32 EEC (OJ No L 154, 5.6.1992, p.1) amending Council Directive 67/548/EEC**	**Official use only**
2.10.1 Human exposure towards active substance	It is understood that the requirement of a full risk assessment for fipronil production and formulation is outside the scope of the Biocide Directive 98/98/EC. Production and work place safety are covered by other local legislation than the BPD. However, some generic information on the production and formulation phases can be found below.	
2.10.1.1 Production		
Production of the active substance		
i) Description of process	Multi-phase chemical synthesis. The final intermediate is oxidised by hydrogen peroxyde. Fipronil is then, crystallized, washed and dried under vacuum. Isolated technical grade fipronil is moistured with water to produce the wet fipronil, which is the manufacturing use product shipped to formulation sites. Due to the confidential nature of this data more information on the manufacturing process can be found in the Business Confidential Information document IIIA-2.6 BCI.	
ii) Workplace description	Fipronil is manufactured in a dedicated plant located XXXXrunning under the national XXXX regulation. Fipronil is produced in a dedicated workshop in batch operations. The annual amount of fipronil necessary for the PT 18 use described in this dossier represents less than 1% of a batch produced in one day. Fipronil is always kept in a confined area (in reactors, pipes) meaning that there is no fipronil in non-confined areas. Access of personnel to this dedicated workshop is strictly controlled. In the whole plant the floor is resin-coated. Production-site personnel working outside of the confined Fipronil production area wear standard protective equipment at all times. This standard equipment is composed of: - protective glasses, - coverall: Tywek, - two pairs of gloves (nitrile), - cover shoes and cover boots.	

Section 2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32 EEC (OJ No L 154, 5.6.1992, p.1) amending Council Directive 67/548/EEC**	**Official use only**
	In the confined zone the protective equipment is dependent on the activity: <u>Level 1:</u> Tasks requiring the presence within the confined zone for short periods of time (typically not more than 15 min/day) and <u>NOT</u> involving the manipulation of the product, i.e., to operate on floodgates Standard equipment plus … - 1 additional pair of surboots, - a 3rd pair of gloves, - a 2nd tyvek coverall, - a mask with a specific cartridge (type P3) <u>Level 2:</u> Tasks requiring the presence within the confined zone for short periods of time (typically not more than 15 min/day) involving the manipulation of the product, i.e., to clean or to set the carousel - Level 1 equipment but with ventilated Helmet instead of the mask. <u>Level 3:</u> Tasks requiring the presence within the confined zone for extended periods of time (no time limitation) involving the manipulation of the product, i.e. cleaning, maintenance - Standard equipment + ventilated tight coveralls.	
iii) Inhalation exposure	Given the low vapour pressure of fipronil (3.9×10^{-7} Pa) and its high melting point (195 – 203°C) there is little potential for exposure through inhalation of vapour. There is no inhalation exposure to particulate matter during the synthetic stage as the process is entirely enclosed. Inhalation of particulate dust during packaging is minimised through automated operations and engineering control (filtered environment) as well as the use of full personal protective equipment.	
iv) Dermal exposure	There is no dermal exposure to particulate matter during the synthetic stage as the process is entirely enclosed. Dermal exposure of particulate dust during packaging is minimised through automated operations and engineering control (filtered environment) as well as the use of full personal protective clothing.	
<u>Formulation</u> of biocidal product		
i) Description of process	A mixture is prepared in a paste blender or kneader containing bait material and gel building substances to which a technical concentrate containing fipronil is added. The gel is homogenised; it has a fipronil content of 0.05 %. Batches of 100 – 500 kg are produced. The gel is filled into cartridges and packed. Due to the confidential nature of this data more information on the formulation process can be provided by the producer upon request.	

Section 2.10 Annex Point IIA, II.2.10	**Exposure data in conformity with Annex VIIA to Council Directive 92/32 EEC (OJ No L 154, 5.6.1992, p.1) amending Council Directive 67/548/EEC**	**Official use only**
ii) Workplace description	Goliath Gel is produced in a plant XXXX in XXXX running under local regulation. In the plant, operator wears personnal protective equipment all the time as required by the local regulation. The technical concentrate is produced 1 – 2 times per year. The vessel is closed and opened only for adding the components. While handling the technical fipronil, specific protective equipment such as a repiratory protection is used in addition to gloves, safety glasses and protective clothings. All materials are handled under a dust collection system. The gel formulation is done by campaigns. The kneader for the gel formulation is closed and opened only for adding the components. The formulator wears working clothes, safety glasses and gloves. All materials are handled under an aspiration system. The filling and packing of cartouches takes place by campaigns. Most of the equipment is mobile and cleaning can take place with water under pressure in a dedicated cleaning room. The cleaning water is collected and disposed as described in the "waste disposal" section below.	
iii) Inhalation exposure	Inhalation during the production of the technical concentrate is minimized by engineering control (air ventilation and air filtration) and personal protective equipments including respiratory protection.	
iv) Dermal exposure	For all activities where skin contact can occur workers wear personnal protective clothings and gloves.	
2.10.1.2 Intended use(s)		
1. Professional Users	Human exposure resulting from intended uses is considered to be product-related. Thus, exposure estimates are provided in Document III-B, Section 6.6 (Information related to the exposure of the biocidal product).	
2. Non-professional Users including the general public	Not relevant, fipronil-based biocidal products are intended for professional use only	
2.10.2 Environmental exposure towards active substance	Production and formulation stages for Goliath Gel operate in compliance with appropriate national regulations.	

Section 2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32 EEC (OJ No L 154, 5.6.1992, p.1) amending Council Directive 67/548/EEC**	**Official use only**
2.10.2.1 Production		
<u>Production</u> of the active substance		
(i) Releases into water	Maintenance and cleaning operations are always made by operators who are regularly trained to respect the specific safety principles. Cleaning water is then disposed as described in the "Waste disposal" section described below. Effluents are contained in specific retention basins and then they are treated according to the national authorisation	
(ii) Releases into air	As described fipronil is always kept in confined area. The bagging phase is secured by using automatic systems and there is no air exhausted in the environment. Solid wastes are then disposed as described in the "Waste disposal" section below.	
(iii) Waste disposal	Solid and liquid wastes are disposed by incineration in a dedicated plant. Therefore no release in the environment can be expected from the waste disposal.	
<u>Formulation</u> of biocidal product		
(i) Releases into water	There is no release into water. There is no water involved in the production process. Furthermore, there is a dedicated mixing vessel used only for the Fipronil premix to minimize waste water. Cleaning water and potential effluents are collected and disposed as described in the "waste disposal section" below.	
(ii) Releases into air	Air dust collection systems are installed where the technical Fipronil is handled. The filtered air is recycled within the plant. There is no air exhaust to the environment.	
(iii) Waste disposal	Both solid waste and cleaning water are disposed by incineration in a specialized plant. Therefore there is no release of fipronil in the environment.	
2.10.2.2 Intended use(s)		
Affected compartment(s):	Environmental exposure resulting from intended uses is considered to be product-related. Thus, exposure estimates are provided in the attached document to Doc IIC: Environmental Risk Assessment for Goliath Gel, CEA.115.	**X**
water	Please see the attached document to Doc IIC: Environmental Risk Assessment for Goliath Gel,XXXX.	**X**
sediment	Please see the attached document to Doc IIC: Environmental Risk Assessment for Goliath Gel, XXXX.	**X**
air	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, XXXX.	**X**

Section 2.10 **Annex Point IIA, II.2.10**	**Exposure data in conformity with Annex VIIA to Council Directive 92/32 EEC (OJ No L 154, 5.6.1992, p.1) amending Council Directive 67/548/EEC**	**Official use only**
soil	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, CEA.115.	**X**
Predicted concentration in the affected compartment(s)		
water	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, XXXX.	**X**
sediment	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, XXXX.	**X**
air	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, XXXX.	**X**
soil	Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, XXXX.	**X**

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	2.10.2 Environmental exposure towards active substance 2.10.2.2 Intended use(s) ~~*Please see the attached document to Doc IIC : Environmental Risk Assessment for Goliath Gel, CEA.115.*~~ Due to the very specific intended use of Goliath Gel indoors, the emissions of fipronil to surface water, sediments and soil are considered as very limited (according to the report CEA.115 : Environmental Risk Assessment for Goliath Gel, Mason 2006)(see also Doc II Section C13).
Conclusion	Adopt applicant's version with above amendment.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.1 Melting point, boiling point, relative density (IIA3.1)								
3.1.1 Melting point								
Melting pt. 1	OECD 102	Fipronil substance, pure (99.7 %)	***Result***: 204.1 – 204.5°C ***Pressure:*** atmospheric		Y	1	XXXX Daum, A. 2004	
Melting pt. 2	EU (=EEC) 92/69 A.1	Fipronil substance, pure (99.3% w/w)	***Result:*** 203°C Pressure: atmospheric		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	
Melting pt. 3	EU (=EEC) 92/69 A.1	Fipronil substance, technical (96.6% w/w)	***Result:*** 195 – 203°C Pressure: atmospheric		Y	1	XXXX Chabassol, Y.C. Hunt, G.M. 1991x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.1.2 Boiling point								
Boiling pt. 1	OECD 102	Fipronil substance, pure (99.7 %)	At ca. 220°C decom-position started indicated by an exo-thermic effect and gas evolutuíon at 238 °C. No boiling point was observed.		Y	1	XXXX Daum, A. 2004	
Boiling pt. 2	EU (=EEC) 92/69 A.1	Fipronil substance, pure (99.3% w/w)	Not strictly required as the product is a solid with a high melting point. The substance therefore has no boiling point at atmospheric pressure		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
Boiling pt. 3	EU (=EEC) 92/69 A.1	Fipronil substance, pure (99.3% w/w)	There is an exothermal decomposition of pure fipronil starting at 230°C		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	
3.1.3 Bulk density/relative density								
Bulk/Rel. density 1	EU (=EEC) 92/69 A.3 OECD 109	Fipronil substance, pure (99.8% w/w)	The relative density of pure fipronil is $D^R_4 = 1.705$		Y	1	XXXX Nobuhiro, K. 2001x	**X**
Bulk/Rel. density 2								
3.2 Vapour pressure (IIA3.2)								
Vapour pressure 1	EEC A.4 OECD 104	Fipronil substance, pure (99.8% w/w)	**Temperature:** 25°C Result: 2.0×10^{-6} Pa Temperature: 50°C Result: 3.5×10^{-5} Pa		Y	1	XXXX Nobuhiro, K. 2001x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
Vapour pressure 2	OECD 104	Fipronil substance, technical (95.2 % w/w)	**Temperature:** 25°C Result: 3.7 x 10^{-7} Pa		Y	1	XXXX Chabassol, Y. Reynaud, R. 1991x	**X**
3.2.1 Henry´s Law Constant (Pt. I-A3.2)								
Henry´s Law Constant 1	Calculation	Fipronil substance, pure	The Henry's constant at 25°C using data from purified material was calculated to be: 2.31 x 10^{-4} Pa.m^3.mol^{-1}		n.a	1	XXXX Bascou, J.P. 2002x	**X**
Henry´s Law Constant 2	Calculation	Fipronil substance, technical	The Henry's constant at 20°C using data from technical material was calculated to be: 3.75 x 10^{-5} pa.m^3.mol^{-1}		n.a	1	XXXX Chabassol, Y. 1992x	
3.3 Appearance (IIA3.3)								

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.3.1 Physical state	USEPA (=EPA) OPPTS 830.6303	Fipronil substance, technical (96.6 % w/w)	Powder		Y	1	XXXX Chabassol, Y. Hunt, G.M. 1991x	**X**
3.3.2 Colour	USEPA (=EPA) OPPTS 830.6303	Fipronil substance, technical (96.6 % w/w)	White		Y	1	XXXX Chabassol, Y. Hunt, G.M. 1991x	**X**
3.3.3 Odour	USEPA (=EPA) OPPTS 830.6303	Fipronil substance, technical (97.4 % w/w)	Mouldy smell		Y	1	XXXX Chabassol, Y. Hunt, G.M. 1991x	**X**

Section A3	**Physical and chemical properties of active substance**
Annex Point IIA, III	

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.4 Absorption spectra (IIA3.4)								
UV/VIS	OECD 101	Fipronil substance, pure (99.7% w/w) Code no. AE F124964 00 1B99 0002	Molar extinction coefficients of the UV/VIS absorption maxima in methanolic solution: ε = 48385 L.mol^{-1}.cm^{-1} (λ= 203nm) ε = 7457 L.mol^{-1}.cm^{-1} (λ= 279nm) ε = 7281 L.mol^{-1}.cm^{-1} (λ= 286nm) Molar extinction coefficient at a wavelength above 290nm ε = 6008 L.mol^{-1}.cm^{-1} (λ= 291nm)	These spectral data are in agreement with the chemical structure	Y	1	XXXX Muehlberger, B. 2001x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
IR	Infra-red absorption spectrum	Fipronil substance, pure (99.3% w/w) Batch No. AJK232	WAVE NUMBER (cm^{-1}) — ASSIGNMENT 3438 – 3328 — NH_2 stretching 2256 — CN stretching 1633 — NH_2 bending 1324 – 1145 — $ArCF_3$ stretching 1203 — CF_3 stretching		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	
NMR	1H NMR spectrum	Fipronil substance, pure (99.3% w/w) Batch No. AJK232	Chemical shifts * (ppm) — assignment 7.84, sharp singlet, 2H — aromatic H 5.18, broad singlet, 2H — NH_2 * reference TMS, solvent $CDCl_3$		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	
	19F NMR spectrum	Fipronil substance, pure (99.3% w/w) Batch No. AJK232	Chemical shifts * (ppm) — assignment -63.9, singlet, 3F — aromatic C**F**$_3$ -75.4, singlet, 3F — sulfinyl C**F**$_3$ * reference $CFCl_3$, solvent $CDCl_3$		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
	13C NMR spectrum	Fipronil substance, pure (99.3% w/w) Batch No. AJK232	Chemical shifts * assignment (ppm) 150.2 pyrazole **C**-NH_2 136.5 aryl **C**-Cl (2) 135.4 aryl **C**-CF_3 126.4 aryl **C**-H (2) 125.8 pyrazole **C**-CN 124.8 aryl **C**F_3 121.7 S-**C**F3 110.1 **C**N 93.2 pyrazole **C**-s * reference TMS, solvent $CDCl_3$		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	
MS	Mass Spectrum (EI)	Fipronil substance, pure (99.3% w/w) Batch No. AJK232	m/z assignment 436 (2 ^{35}Cl) molecular ion M+ 367 (2 ^{35}Cl) base peak (M-CF_3)+ 213 (2 ^{35}Cl) {$ArCl_2CF_3$}+		Y	1	XXXX Chabert, M.S. Lecourt, N.C. 1996x	

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.5 Solubility in water (IIA3.5)								
Water solubity 1	EEC A. 6 OECD 105	Fipronil substance, pure (98.9 % w/w)	**Results: (20 °C)** 5.84 mg/l in deionized water 5.29 mg/l @ pH 4 3.35 mg/l @pH 7 3.97 mg/l @ pH 9		Y	1	XXXX Daum, A. 2005	**X**
Water solubility 2	EEC A.6 OECD 105	Fipronil substance, pure (99.3% w/w)	**result:** 3.78 mg/l **temperature:** 20°C **pH:** 6.58		Y	1	XXXX Nobuhiro, K. 2001x	
Water solubility 3	EEC A.6 OECD 105	Fipronil substance, technical (95.4% w/w)	2.4 mg/l @ pH 5 @ 20°C 1.9 mg/l @ pH 7 @ 20°C 2.2 mg/l @ pH 9 @ 20°C		Y	1	XXXX Chabassol, Y.C. Reynaud, R. 1991x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.6 Dissociation constant (-)	OECD 112	Fipronil substance, pure (99.3% w/w)	All three methods of OECD 112 to determine dissociation constants (the titration method, the spectrophotometric method, and the conductometric method) are not suitable for the determination of the dissociation constant (pK_a) of Fipronil		N.a	1	XXXX Cichy, M. 2001x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA3.1)	EU (=EEC) 94/37	Fipronil substance, technical (96.7% w/w)	solvent g/100ml acetone 54.59 methylene chloride 2.23 ethyl acetate 26.49 n-hexane 0.0028 methanol 13.75 1-octanol 1.22 2-propanol 3.62 toluene 0.3 Effect of temperature: The effect of the temperature on solubility was not assessed in the report however, the solubility of fipronil in organic solvents was determined by using the CIPAC method MT 157 Part 2 (Flask Method) which is a standard method widely accepted by Regulatory Authorities. A saturated solution of fipronil in organic solvent was prepared and stirred for at least 24h at a minimal temperature of 30°C ; then, the solution was allowed to re-equilibrate for 24h at 20 °C before determination of the solubility.		Y	1	XXXX Chabassol, Y.C. Reynaud, R. 1991x	**X**

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA3.2)				As manufactured, the active ingredient does not include an organic solvent				
3.9 Partition coefficient n-octanol/water (IIA3.6)								
log Pow 1	OECD 107	Fipronil substance, pure (99.3% w/w)	**result**: 4.0 **temperature**: 20°C **pH**: not recorded	Shake flask method	Y	1	XXXX Chabassol, Y. Reynaud, R. 1991x	
log Pow 2	EEC A8 OECD 107	Fipronil substance, pure (99.9% w/w)	**result:** 3.5 **temperature:** 20°C **pH**: not recorded	HPLC method. Value consistent with the above	Y	1	XXXX Cousin, J. 1997x	

Section A3 **Physical and chemical properties of active substance** **Annex Point IIA, III**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
log Pow 3 Effect of pH	n.a.	n.a		pH value is not expected to affect log P value significantly. No pKa can be established for the molecule (See3.6). In addition, the solubility at different pHs in water does not vary significantly (see 3.5) and the molecule is not expected to ionize at biological pHs	n.a	n.a	n.a	
3.10 Thermal stability, identity of relevant breakdown products (IIA3.7)	OECD 102	Fipronil substance, pure (99.7 %)	There is no degradation up to the melting point (203.8 °C)		Y	1	XXXX Daum, A. 2004	

Section A3 **Physical and chemical properties of active substance**
Annex Point IIA, III

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.11 Flammability, including auto-flammability and identity of combustion products (IIA3.8)								
flammability	EU (=EEC) 92/69 A10	Fipronil substance, technical (96.2% w/w)	Not highly flammable up to 200°C (sample melted)		Y	1	XXXX Cousin, J. Fillion, J. 1996x	
Auto-flammability	EU (=EEC) 92/69 A16	Fipronil substance, technical (96.2% w/w)	Not auto flammable up to 200°C (sample melted)		Y	1	XXXX Cousin, J. Fillion, J. 1996x	
3.12 Flash-point (IIA3.9)								
Flash-point 1				Not required – as melting point is >40 °C				

Section A3 **Annex Point IIA, III**	**Physical and chemical properties of active substance**

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
3.13 Surface tension (IIA3.10)								
Surface tension 1	84/449/EWG, A.5 OECD 115 DIN 53914	Fipronil substance, technical (96.2% w/w)	**result:** 72.5 mN/m **temperature**: 20 °C **concentration**: 1g/l in distilled water		Y	1	XXXX Cousin, J. 1996x	**X**
3.14 Viscosity (-)			**Result:** Temperature:	Not required – as material is not liquid				
3.15 Explosive properties (IIA3.11)								
	VDI Guideline 2263 UN Transport of Dangerous Goods Chapter 14 EU (=EEC) 92/69 A14	Fipronil substance, technical (96.2% w/w)	Lower explosion limit = 30 g/m^3 lowest minimum ignition energy >10J Fipronil shows no auto ignition		No	2 – Non GLP study	XXXX Vandermarliere, P. 1992x	**X**

Section A3 **Physical and chemical properties of active substance**
Annex Point IIA, III

Subsection (Reference)	**Method**	**Purity/ Specification**	**Results**	**Remarks/ Justification**	**GLP (Y/N)**	**Reliability**	**Reference**	**Official use only**
	VDI Guideline 2263 UN Transport of Dangerous Goods Chapter 14 EU (=EEC) 92/69 A14	Fipronil substance, technical (96.2% w/w)	Fipronil technical does not show a danger of explosion		Y	1	XXXX Tran Thanh Phong, J. 1999a	**X**
3.16 Oxidizing properties (IIA3.12)	84/449/EWG, A17	Fipronil substance, technical (96.1%, lot no. OP9850229; 95.7%, lot no. OP9850225)	Fipronil has no oxidizing properties	The study was performed using two batches of technical active substance.	Y	1	XXXX Tran Thanh Phong, J. 1999a	**X**
3.17 Reactivity towards container material (IIA3.13)	US-EPA, 40 CFR158 Subpart C, Subdivision D, series 63.17	Fipronil substance, technical, wet MUP, 95.2 %	During manufacturing handling or storage, corrosiveness of fipronil on packaging material, containers or apparatus was never observed. Storage stability data on wet fipronil indicated no reactivity towards container material (polyethylene).		Y	1	XXXX Cousin, J. 1997	

Section A3 Physical and Chemical Properties of active substance

Annex point IIA, III

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*Revised January 2010*
Materials and methods	**Revision/amendments:**

3.1.1 Melting point

Melting point 3 : the melting point was measured on 2 products of different purity : 96.6% and 97.4% w/w, which gave respectively 195°C and 203°C.

3.1.2 Boiling point

Boiling point 2 and 3 refer to the same study (Chabert,.Lecourt, 1996). Keep only one line.

3.1.3 Bulk density/relative density

Temperature of the measurement should be stated : ~~D^R_4~~ D^{20}_4

3.2 Vapour pressure

Vapour pressure 1

Purity of the test article was 99.4%, not 99.8%.

The result at 25°C is a limit value ($\leq 2.0 \times 10^{-6}$ Pa), which can be accepted because melting point is above 200°C.

Vapour pressure 2

The result at 25°C was calculated from measures at 50°C (1.7×10^{-5} Pa) and 35°C (0.2×10^{-5} Pa).

3.2.1 Henry´s Law constant

Henry´s Law Constant 1

This value is not correct since it calculated from a limit value (see 3.2/01). However it can be kept as a limit value: $< 2.31 \times 10^{-4}$ Pa.m^3.mol^{-1}. Reliability is 2.

Purity is 99.4%.

3.3.1 Physical state; 3.3.2 Colour; 3.3.3 Odor

Samples of two purities were tested : *96.6 % and 97.4 % w/w*

Add results for purified Fipronil (99.3%) from study A3.9/01(*XXXX*; Chabassol, Y., Reynaud, R.;1991x): white, powder, odorless.

3.4 Absorption spectra

UV/VIS : No peak at 291nm but absorption was determined in accordance with the requirement for Annex point IIA 2.5.1 of 91/414/EC Directive.

3.5 Solubility in water

Water solubitliy 1**:** *5.84 mg/l in deionized water* (pH 5.7)

Water solubility 3: *1.9 mg/l @* ~~*pH 7*~~ in distilled water (pH 6) *@ 20°C*

2.6 mg/l in distilled water (pH 6.5) @ 10°C

The influence of temperature on solubility is not fully studied (the value at 30°C is missing) but the value at 10°C is accepted to show that the solubility in water is

Section A3 Physical and Chemical Properties of active substance

Annex point IIA, III

not temperature dependant.

3.6 Dissociation constant

The dissociation constant was not determined, but the study shows that the methods from OCDE 112 are not suitable.
The study is not GLP. Reliability indicator is 2.

3.7 Solubility in organic solvents

The method is CIPAC MT 157 ~~*EU (=EEC) 94/37*~~

The justification of non-submission on the influence of temperature on solubility is not acceptable.

The non-submission is justified by :

A relative increase the solubility of Fipronil is expected with increased temperature, but no significant changes are expected: the values will stay in the same ranges.

3.13 Surface tension

The method is not ~~*84/449/EWG*~~ but,EU (=EEC) 92/69

The concentration is not ~~*1g/l*~~ but 90% of saturation : about 2 mg/l in distilled water.

3.15 Explosive properties

The values of lower explosion limit and lowest minimum ignition energy are not required. However they give important information about explosibility of dust clouds. The method is VDI 2263.

The information on auto-ignition (method from UN Transport of Dangerous Goods 14.3.4) is not required. However it can be kept and placed in section 3.11.

The method for the evaluation of explosion properties is EC 92/69 A14.

3.16 Oxidizing properties

The method for the evaluation of oxidizing properties is EC 92/69 A17.

Conclusion	Adopt applicant´s version
Reliability	As indicated in the table (except if amendments)
Acceptability	acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Results and discussion	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant´s summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A4.1 **Annex Point IIA, IV.4.1**		**Analytical methods for the determination of pure active substance**	
		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.1/01** Robles, J.M, and Cousin, J. (1996) Technical Fipronil HPLC determination of active ingredient. Rhône-Poulenc Secteur Agro, Anal Method No. F-735-06-96, 18 July 1996. 10 pages (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Where appropriate grind and homogenise sample. Dissolve about 25mg to 50ml in acetonitrile	
3.1.2	Cleanup	No	
3.2	**Detection**		
3.2.1	Separation method	Isocratic elution HPLC on a reversed stationary phase(Necleosil C18, particle size 5µm; stainless steel, 250mm x 3mmID). Eluate: phosphoric acid (c=0.05 mol/L)/acetonitrile/ methanol = 45/33/22%	
3.2.2	Detector	UV at 220 nm	
3.2.3	Standard(s)	0.5g/l fipronil as external standard	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.4 to 0.6 g/l (80 to 120% of the nominal sample concentration)	
3.3.2	Number of measurements	5	

Section A4.1 **Annex Point IIA, IV.4.1**		**Analytical methods for the determination of pure active substance**	
3.3.3	Linearity	r^2>0.990	
3.4	**Specifity: interfering substances**	The resolution between fipronil and the related compound named 3 carboxylic acid ethyl ester is poor, but is sufficient to show its presence if any. However, this compound has not been found up to now to be present in the technical product	
3.5	**Recovery rates at different levels**	100.3 – 102.1% (mean 101.2%)	x
3.5.1	Relative standard deviation	Standard deviation 0.4%	
3.6	**Limit of determination**	Not applicable	
3.7	**Precision**		
3.7.1	Repeatability	The precision of the method was determined by analysing six sub samples of a batch of technical product. The level of precision was (relative standard deviation) 0.2%	
3.7.2	Independent laboratory validation	Yes	x
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	For validation of the method technical batches of fipronil were used. Pure fipronil material served as reference substance. All standard and sample solutions were prepared as described in the analytical method. Standard solutions for the linearity determinations ranged from 80 to 120% of the nominal concentration of fipronil in the technical material Specificity: Certified reference materials of fipronil and organic impurities were run to the method conditions to determine any co-elutions. Linearity over an appropriate working range: 5 standard levels were made up spanning the range 80 to 120% of the nominal sample concentration. Accuracy: The accuracy was estimated by comparing the known amounts of fipronil weighed in the calibration solutions used for the repeatability test with amounts calculated by using the peak areas measured for three solutions and the linear regression equatation of the linearity test. Precision: 5 replicate determinations of a technical batch were performed.	x

Section A4.1 **Annex Point IIA, IV.4.1**		**Analytical methods for the determination of pure active substance**	
4.2	**Conclusion**	Specificity: Sufficient separation of impurities, eluent and active substance is demonstrated by the chromatograms. No chromatographic interference between fipronil and significant impurities. Linearity over an appropriate working range: Linearity of the calibration curve was observed from 80 to 120% w/w (of the nominal sample concentration) corresponding to 0.4 to 0.5 g/l (working range). The correlation coefficient was calculated to be r^2>0.990. Accuracy: The method was estimated to be accurate since the recovery percentage obtained was over the range 100 to 120% Precision: the repeatability, expressed as coefficient of variation, according to the validation was CV = 0.2% (relative).	
4.2.1	Reliability	1	
4.2.2	Deficiencies	No	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised April 2010
Materials and methods	**3.5 Recovery rates at different levels** Recovery rates were calculated from 14 measurements. **3.7.2 Independent laboratory validation** No information in the report shows that the method was validated by an independent laboratory. **4.1 Materials and methods** *Accuracy: The accuracy was estimated by comparing the known amounts of fipronil weighed in the calibration solutions used for the repeatability test with amounts calculated by using the peak areas measured for ~~three~~ these solutions and the linear regression ~~equatation~~ equation of the linearity test.* *Precision: ~~5~~ 6 replicate determinations of a technical batch were performed.* **4.2 Conclusion** *Linearity over an appropriate working range: Linearity of the calibration curve was observed from 80 to 120% w/w (of the nominal sample concentration) corresponding to 0.4 to ~~0.5~~ 0.6 g/l (working range).*
Conclusion	Adopt applicant's version with the above amendments.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Au Section A4.2 **Annex Point IIA, IV.4.2**	**Analytical methods: soil, air, water, animal and human body fluids and tissues**

Section A4.2.1		**Soil**	
		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.1/01** Ballesteros, C., Claviere, B. and Kieken, J.-L. (2000) Fipronil and its metabolites (XXXX): Analytical method for the determination of residues in 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC/96/46	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Residues are extracted from the soil samples by macerating with a mixture of acetonitrile and water.	
3.1.2	Cleanup	The extract is purified using a polystyrene-divinylbenzene (HR-P) cartridge	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography	**X**
3.2.2	Detector	Electron Capture Detector or a Mass Spectrometric Detector	**X**
3.2.3	Standard(s)	Well characterised reference standards of each analyte	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	For each analyte detector response for GC/EC analysis 1.0 – 20µg/l For each analyte detector response for GC/MSD/Cl^- analysis 0.02 – 0.2 µg/l	
3.3.2	Number of measurements	5 for each analyte (GC/EC analysis)	**X**

Section A4.2.1		**Soil**	
3.3.3	Linearity	Correlation coefficient (GC/EC analysis) fipronil r^2>0.9973 XXXX r^2>0.9991 XXXX r^2>0.9993 XXXX r^2>0.9988	**X**
3.4	**Specificity: interfering substances**	The specificity is assured by the sample preparation procedure and the chromatographic analysis. Reagent blank samples were analysed with GC/MSD/CI^- analysis. For each compound, the interferences due to the reagents were estimated to be lower than 20% of the limit of quantification (LOQ). In control soil samples the interferences were < 30% LOQ. Only in one control sample interference from XXXX was noted.	
3.5	**Recovery rates at different levels**	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% See Tables A4.2.1.1-1 to A4.2.1.1-3	
3.5.1	Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% See Tables A4.2.1.1-1 to A4.2.1.1-3	
3.6	**Limit of determination**	LOQ = 0.002 mg/kg for each analyte	
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1	
3.7.2	Independent laboratory validation	Not required	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Residues were extracted from the soil samples by macerating with a mixture of acetonitrile and water. The extract was purified using a polystyrene-divinylbenzene (HR-P) cartridge. The quantification was carried out by Gas Chromatography (GC) using an Electron Capture Detector (ECD) or a Mass Spectrometric Detector (MSD) using negative chemical ionisation CI^- mode (MSD/ CI^-). The quantification was done by external standardisation..	

Section A4.2.1	**Soil**	
4.2 Conclusion	Specificity: Fipronil and its metabolites (XXXX) can be determined due to the specific detection techniques. Repeatability: See results of RSD (Relative Standard Deviation) in Tables A4.2.1.1-1 to A4.2.1.1-3 Limit of Quantification (LOQ): 0.002 mg/kg for each product Confirmatory method: Yes, use of a mass selective detector (MSD) Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification as well as ten times this limit. See Tables A4.2.1.1-1 to A4.2.1.1-3 **Conclusion**: The method referenced in this section was successfully validated in different European soil types.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.2.1.1-1

Chazay (France)				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< LOQ (n=5)	< LOQ (n=5)	< LOQ (n=5) LOQ (n=1)(c)	< LOQ (n=5)
0.002 mg/kg	102; 94; 108 109; 95; 92 77(a); 84(a); 93(a) Mean: 95% RSD: 10%	109; 101; 103 120; 100; 103 95(a); 96(a); 99(a) Mean: 103% RSD: 7%	97; 90; 97 108; 89; 89 100(b); 80(b); 120(b) Mean: 97% RSD: 12%	95; 87; 90 100; 85; 88 80(a); 78(a); 84(a) Mean: 87% RSD: 8%
0.020 mg/kg	71; 84; 88 89(a); 74(a); 91(a) Mean: 83% RSD: 9%	78; 94; 101 82(a); 63(a) 83(a) Mean: 84% RSD: 14%	76; 95; 97 91(a); 69(a) 98(a) Mean: 88% RSD: 13%	78; 93; 97 80(a); 68(a) 81(a) Mean: 83% RSD: 12%

(a): analysis with MSD/CI^- detection
(b): analysis with MSD/CI^- detection, value obtained after deduction of the interfering peak identified as XXXX by GC-MSD/ CI^-
(c): pollution with XXXX during field treatment

Table A4.2.1.1-2

Neuville St Vaast (France)				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< 30%LOQ (n=3)	< 30%LOQ (n=3)	< 30%LOQ (n=3)	< 30%LOQ (n=3)
0.002 mg/kg	80(a); 74(a) 84(a); 85(a) 110(a); 83(a) Mean: 86% RSD: 13%	81(a); 66(a) 83(a);75(a) 103(a); 77(a) Mean: 81% RSD: 14%	101(a); 76(a) 89(a); 83(a); 118(a); 82(a) Mean: 91% RSD: 16%	78(a); 69(a) 84(a); 76(a) 105(a); 82(a) Mean: 82% RSD: 14%
0.020 mg/kg	81; 74; 107; 103 84; 73; 107; 91; 81 Mean: 89% RSD: 14%	79; 77; 102 94; 88; 71 107; 84; 71 Mean: 86% RSD: 14%	94; 90; 127; 115 98; 80; 127; 97 97; 83 Mean: 101% RSD: 17%	78; 76; 102 95; 84; 72 ; 106; 88; 72 Mean: 86% RSD: 14%

(a): analysed with MSD/CI⁻ detection

Table A4.2.1.1-3

Bologna (Italy)				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< LOQ (n=2)	< LOQ (n=2)	< LOQ (n=2)	< LOQ (n=2)
0.002 mg/kg	100; 82; 78 109; 114; 93 Mean: 96% RSD: 14%	110; 88; 82 115; 124; 100 Mean: 103% RSD: 14%	97; 77; 77 114; 122; 92 Mean: 97% RSD: 18%	91; 79; 70 106; 113; 89 Mean: 91% RSD: 16%
0.020 mg/kg	84; 62; 82 Mean: 76% RSD: 16%	94; 67; 82 Mean: 81% RSD: 17%	92; 66; 75 Mean: 78% RSD: 17%	94; 66; 87 Mean: 82% RSD: 18%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	***Revisions/amendments :*** 3.2.1 Separation method The details on the chromatographic method are missing: GC/ECD: - The column is Rtx®-1701 Crossbond® (14% cyanopropylphenyl-86% dimethylpolysiloxane), 15 m, 0.530 mm i.d., 0.250 µm film thickness) - Carrier gas: helium, 10 mL/min - Temperature programme: 90°C hold 0.5 min then 30°C/min to 220°C, 10°C/min to 240°C hold 4 min and 10°C/min to 280°C hold 10 min - Injection: 1 µL, 90°C hold 0.25 min then 180°C/min to 240°C hold 20 min GC/MSD: - The column is DB-XLB (30 m, 0.320 mm i.d., 0.500 µm film thickness) - Temperature programme: 50°C hold 1.5 min then 25°C/min to 250°C hold 5 min - Injection: 1 µL, 50°C hold 0.5 min then 1°C/min to 70°C hold 1.5 min and 6°C/min to 260°C hold 10 min, 3.2.2 Detector *Electron Capture Detector* (EC) *or a Mass Spectrometric Detector* (MSD) Add the type of ionization for MSD: Ionization mode: negative chemical ionization (CI^-) 3.3.2 Number of measurements *5 for each analyte (GC/EC analysis*) 8 for each analyte (GC/MSD/CI^- analysis) 3.3.3 Linearity add: Correlation coefficient (GC/MSD/CI^- analysis) fipronil $r^2>0.9962$ XXXX $r^2>0.9978$ XXXX $r^2>0.9969$ XXXX $r^2>0.9975$
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	The metabolite XXXX is not studied in this method, but it is in the next method (A4.2.1/02)
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	

Reliability
Acceptability
Remarks

Section A4.2.1	**Soil**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**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. XXXX; 16 September 2005 ; 61 pages (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 91/414; EEC 96/46; SANCO/825/00 rev. 6 of 20 June 2000; SANCO/3029/99 rev. 4 (11 July 2000); EPA 850.7100	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Extraction with methanol/water	
3.1.2	Cleanup	No	
3.2	**Detection**		
3.2.1	Separation method	LC-MS/MS	**x**
3.2.2	Detector	Mass Spectrometric Detector	**x**
3.2.3	Standard(s)	Well characterised reference standards of each analyte	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	10, 20, 50, 100 and 200 pg/ml	
3.3.2	Number of measurements	5 for each analyte	

Section A4.2.1	**Soil**	
3.3.3 Linearity	Correlation coefficient fipronil r^2>0.9998 XXXX r^2>0.9992 XXXX r^2>0.9994 XXXX r^2>0.9994 XXXX r^2>0.9986	
3.4 Specifity: interfering substances	No interferences	
3.5 Recovery rates at different levels	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% See Tables A4.2.1.2-1 to A4.2.1.2-2	**x**
3.5.1 Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% See Tables A4.2.1.2-1 to A4.2.1.2-2	
3.6 Limit of determination	LOQ = 0.002 mg/kg in soil for each compound	
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	A 5 g sample aliquot is extracted with 50 mL methanol/water on a mechanical shaker. Approx. 5 mL of the suspension is transferred into a 10 mL centrifuge tube and centrifuged at 4000 rpm (5 min, 20°C). The extract is diluted with the appropriate amount of methanol/water for LC- MS/MS measurement. Confirmatory method: Due to the high selectivity and specificity of LC-MS/MS an additional confirmatory technique is not necessary. However, recoveries have also been determined for all analytes based on a second mass transition. The results of both transitions were in very good agreement.	

Section A4.2.1	**Soil**	

4.2	**Conclusion**	Fipronil and its metabolites XXXX were determined in fortified soil samples of two different types (loamy sand and sandy loam). The tested fortification levels were 0.002, 0.02 and 0.2 mg/kg. All mean recovery values (5 replicates each) are in the range 98.2-111.5%. Only the mean recovery value of XXXX in soil 2.2 at fortification 0.002 mg/kg (LOQ) slightly exceeds the upper EU guidance limit of 110 %. However, due to the good repeatability this value (111.5%) is considered acceptable. For each fortification level the %RSD values are <20% (range: 1.1-8.3 %). Recoveries and repeatability data are shown below. The tested untreated soil samples showed no significant interferences at the retention times of Fipronil, XXXX indicating adequate specificity. **Conclusion**: Based on these results method 547/0 is considered valid for the determination of Fipronil, XXXX in soil with a LOQ of 0.002 mg/kg.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

Table A4.2.1.2-1

Soil 2.2					
	Fipronil	**XXXX**	**XXXX**	**XXXX**	**XXXX**
Control value	ND (n=3)	ND (n=3)	ND (n=3)	ND (n=3)	ND (n=3)
0.002 mg/kg	100.8; 99.2 99.3; 98.1 105.2 Mean: 100.5% RSD: 2.8%	104.7; 99.1 101.9; 101.2; 103.3 Mean: 102.0% RSD: 2.1%	103.1; 104.7 105.9;104.4 101.8 Mean: 104.0% RSD: 1.5%	110.9; 113.0 119.4; 106.9 107.2 Mean: 111.5% RSD: 4.6%	100.0; 101.1 107.0; 100.6 101.3 Mean: 102.0% RSD: 2.8%
0.02 mg/kg	104.8; 102.3 101.4; 100.7 103.0 Mean: 102.4% RSD: 1.5%	100.7; 105.3 105.0; 102.9 100.2 Mean: 102.8% RSD: 2.3%	102.7; 104.5 99.5; 100.6 100.4 Mean: 101.5% RSD: 2.0%	110.8; 103.5 100.7; 101.2 97.2 Mean: 102.7% RSD: 4.9%	107.0; 102.8 101.6; 102.6 101.4 Mean: 103.1% RSD: 2.2%
0.2 mg/kg	100.3; 99.4 107.1; 101.6 103.1 Mean: 102.3% RSD: 3.0%	95.7; 98.4 106.8; 98.5 102.2 Mean: 100.3% RSD: 4.3%	96.0; 101.3 100.6; 100.2 99.9 Mean: 99.6% RSD: 2.1%	98.1; 97.7 101.3; 106.7 99.7 Mean: 100.7% RSD: 3.6%	100.0; 100.4 100.7; 99.7 99.5 Mean: 100.1% RSD: 0.5%

(ND: Not detected)

Table A4.2.1.2-2

Soil 2.3					
	Fipronil	**XXXX**	**XXXX**	**XXXX**	**XXXX**
Control value	ND (n=3)	ND (n=3)	ND (n=3)	ND (n=3)	ND (n=3)
0.002 mg/kg	104.8; 101.1 98.6; 101.7 99.8 Mean: 101.2% RSD: 2.3%	104.9; 108.5 105.9; 106.5 98.8 Mean: 104.9% RSD: 3.5%	100.2; 104.5 105.6; 100.9 101.0 Mean: 102.4% RSD: 2.4%	97.0; 100.7 98.0; 104.1 91.1 Mean: 98.2% RSD: 4.9%	104.6; 103.3 102.6; 102.8 101.4 Mean: 102.9% RSD: 1.1%
0.02 mg/kg	105.5; 103.5 103.8; 94.7 101.3 Mean: 101.8% RSD: 4.1%	104.2; 101.4 103.7; 98.7 103.7 Mean: 102.3% RSD: 2.3%	102.7; 99.0 108.4; 96.2 96.1 Mean: 100.5% RSD: 5.2%	106.5; 96.1 101.6; 105.7 108.9 Mean: 103.8% RSD: 4.8%	103.6; 104.9 103.0; 91.9 102.1 Mean: 101.1% RSD: 5.2%
0.2 mg/kg	103.8; 103.5 98.6; 102.2 99.2 Mean: 101.5% RSD: 2.4%	100.6; 102.8 96.8; 102.9 99.1 Mean: 100.4% RSD: 2.6%	101.8; 104.7 97.5; 100.6 97.2 Mean: 100.4% RSD: 3.1%	105.1; 108.4 88.3; 109.5 103.9 Mean: 103.0% RSD: 8.3%	102.9; 100.8 101.7; 99.5 98.1 Mean: 100.6% RSD: 1.9%

(ND: Not detected)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	04.05.2007, revised January 2010
Materials and methods	***Revisions/amendments :*** **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is Betasil C18, 100×2 mm ID, 5 µm - Mobile phase: solvent A is water/formic acid, 1000/1 v/v and solvent B is methanol/formic acid, 1000/1 v/v - The injection is 50 µL (or higher) and the flow rate is 0.6 mL/min **3.2.2 Detector** Add the type of ionization: negative ion mode **3.5 Recovery rates at different levels** *For each compound and at each fortification level, the mean of recoveries was between 70% and 110%.* Only one mean recovery value slightly exceeds the upper EC guidance limit of 110 %. However, due to the good repeatability this value (111.5%) is considered acceptable.
Conclusion	Adopt the applicant's version with above amendments.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.2	**Air**	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	The technical material has extremely low volatility: 3.7×10^{-7} Pa. In addition the biocidal product is not sprayed but applied as a gel bait there would, therefore, be no significant residues in air.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05.04.2007
Materials and methods	
Conclusion	The justification of non-submission is accepted.
Reliability	
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3	**Water**

Section A4.2.3.1	**Drinking water**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.3.1/01** Diot, R. and 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 XXXX 1 March 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	96/46/EC of July 1996, SANCO/825/00 rev. 6 of 20 June 2000	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Water samples are enriched and purified using a fipronil immuno-affinity cartridge	
3.1.2	Cleanup	See above	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography on a capillary column	**X**
3.2.2	Detector	Mass selective detector in the electron impact mode	**X**
3.2.3	Standard(s)	External standards	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.01 to 0.2 µg/l	
3.3.2	Number of measurements	5	

Section A4.2.3.1	**Drinking water**	
3.3.3 Linearity	Correlation coefficient fipronil r^2>0.9924 XXXX r^2>0.9970 XXXX r^2>0.9933 XXXX r^2>0.9984	
3.4 Specifity: interfering substances	For each analyte the interferences due to reagents and the apparent residues in control samples were estimated to be < 10% LOQ.	
3.5 Recovery rates at different levels	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% See Tables A4.2.3.1.1-1 to A4.2.3.1.1-2	
3.5.1 Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% See Tables A4.2.3.1.1-1 to A4.2.3.1.1-2	
3.6 Limit of determination	LOQ: 0.05 µg/l for each analyte (Limit of detection 0.01 µ/l)	
3.7 Precision		
3.7.1 Repeatability	See3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Residues in drinking water were extracted from the samples using a Fipronil immuno-affinity column. Residues were quantified by Gas Chromatography (GC) on a capillary column using a Mass Selective Detector (MSD) in the electron impact mode (EI). Quantification was performed by external standardisation.	
4.2 Conclusion	Specificity: Fipronil and its metabolites (XXXX) can be determined due to the detection technique. The specificity of the method is assured by the sample preparation (use of a Fipronil immuno-affinity column) and the use of a mass selective detector. Repeatability: see results of RSD (Relative Standard Deviation) below Limit of Quantification (LOQ): 0.05 µg/L for each product (Limit of Detection: 0.01 µg/L) Confirmatory method: not required since use of a mass selective detector Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification. Results presented during the method validation are presented in the following tables:	

Section A4.2.3.1	**Drinking water**	
	Conclusions: The method referenced in this section was successfully validated for the following water substrates: - Mineral water at 0.05 µg/L for Fipronil and each of its metabolites - Tap water at 0.05 µg/L for Fipronil and each of its metabolites Additional validation data (0.1 µg/L and 1.0 µg/L) for drinking water are reported in a separate study [see XXXX Yslan F. *et al*. 1998]	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.2.3.1.1-1

Mineral water				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)
0.05 µg/L	113; 111; 104 114; 106 Mean: 110% RSD: 4%	108; 105; 100 104; 96 Mean: 103% RSD: 5%	98; 104; 96 95; 100 Mean: 99% RSD: 4%	99; 100, 97 97; 96 Mean: 98% RSD: 2%

Table A4.2.3.1.1-2

Tap water				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)	< 10% of LOQ (n = 2)
0.05 µg/L	100; 98; 105 98; 98 Mean: 100% RSD: 3%	99; 98; 99 96; 94 Mean: 97% RSD: 2%	96; 96; 96 96; 98 Mean: 96% RSD: 1%	92; 96, 92 94; 95 Mean: 94% RSD: 2%

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April.2007, revised January 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: - The column is Rtx®-5MS RESTEK (15 m, 0.25 mm i.d., 0.25 µm) - Carrier gas: helium, 2.0 ml/min - Temperature programme: 90°C hold 1.5 min then 15°C/min to 280°C hold 2min - Injection: 100 µL, 100°C, 0.3 min **3.2.2 Detector** Add the type of ionization: Ionization mode: Electron Impact (EI+)
Conclusion	Agree with the applicant's version with above amendments.
Reliability	2
Acceptability	Acceptable
Remarks	The method referenced in this section was successfully validated only for concentrations around 0.05 µg/L for Fipronil and its metabolites (XXXX) in drinking water. An other study (A 4.2.3.1/02) with a similar method was validated for concentrations between 0.1 µg/L and 1.0 µg/L.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.1		**Drinking water**	
		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.3.1/02** Bourgade, C., Jendrzejczak, N. and Yslan, F. (1998) Fipronil and its metabolites (XXXX); Analytical method for the determination of residues in drinking water Report no: XXXX April 2, 1998 (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	96/46/EC of July 1996	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Water samples are enriched and purified using a fipronil immuno-affinity cartridge	
3.1.2	Cleanup	See above	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC on a semi-capillary column)	**X**
3.2.2	Detector	Electron Capture Detector	
3.2.3	Standard(s)	External reference standards of each analyte	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	1 to 10 µg/l	
3.3.2	Number of measurements	6	

Section A4.2.3.1		**Drinking water**
3.3.3	Linearity	Correlation coefficient fipronil r^2>0.992496 XXXX r^2>0.995680 XXXX r^2>0.992951 XXXX r^2>0.993317
3.4	**Specificity: interfering substances**	The specificity of the method is answered by the sample preparation (use of a fipronil immuno-affinity column) and the electron capture detector. For each sample type and each analyte the interferences were < 30% LOQ.
3.5	**Recovery rates at different levels**	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% See Tables A4.2.3.1.2-1 to A4.2.3.1.2-2
3.5.1	Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% See Tables A4.2.3.1.2-1 to A4.2.3.1.2-2
3.6	**Limit of determination**	LOQ: 0.1 µg/l for each analyte
3.7	**Precision**	
3.7.1	Repeatability	See 3.5.1
3.7.2	Independent laboratory validation	Not required
		4. APPLICANT'S SUMMARY AND CONCLUSION
4.1	**Materials and methods**	Residues in drinking water were extracted from the samples using a Fipronil immuno-affinity column. Residues were quantified by Gas Chromatography (GC) on a semi-capillary column. Quantification was performed using an Electron Capture Detector (ECD) and external standardisation

Section A4.2.3.1	**Drinking water**

4.2	**Conclusion**	Specificity: Fipronil and its metabolites (XXXX) can be determined due to the detection technique. The specificity of the method is assured by the sample preparation (use of a Fipronil immuno-affinity column) and the detection method. Repeatability: see results of RSD (Relative Standard Deviation) below : Tables A4.2.3.1.2-1 to A4.2.3.1.2-2 Limit of Quantification (LOQ): 0.1 µg/L for each product Confirmatory method: [see XXXX) Diot R., Kieken J.-L. 2002] Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification as well as ten times this limit. Results from the method validation are presented in the following Tables A4.2.3.1.2-1 to A4.2.3.1.2-2. **Conclusion**: The method referenced in this section was successfully validated for the following water substrates: - Mineral water at 0.1 and 1.0 µg/L for Fipronil and each of its metabolites -Tap water at 0.1 and 1.0 µg/L for Fipronil and each of its metabolites.
4.2.1	Reliability	1
4.2.2	Deficiencies	None

Table A4.2.3.1.2-1

Mineral water				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)
0.1 µg/L	100; 105; 101 101; 100 Mean: 101% RSD: 2%	100; 97; 100; 97; 99 Mean: 99% RSD: 2%	108; 103; 105 106; 103 Mean: 105% RSD: 2%	101; 97; 102 102; 103 Mean: 101% RSD: 2%
1.0 µg/L	96; 96; 92 95; 97 Mean: 95% RSD: 2%	94; 89; 93 90; 95 Mean: 92% RSD: 3%	97; 95; 95 92; 97 Mean: 95% RSD: 2%	97; 96; 98 92; 99 Mean: 96% RSD: 3%

Table A4.2.3.1.2-2

Tap water				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)	< 30% of LOQ (n = 2)
0.1 µg/L	97; 105; 113 102; 100 Mean: 103% RSD: 6%	91; 101; 94 99; 98 Mean: 97% RSD: 4%	102; 109; 102 106; 103 Mean: 104% RSD: 3%	100; 107; 95; 105; 103 Mean: 102% RSD: 5%
1.0 µg/L	83; 83; 88 92; 90 Mean: 87% RSD: 5%	77; 75; 83 94; 92 Mean: 84% RSD: 10%	85; 85; 90; 93; 95 Mean: 90% RSD: 5%	84; 91; 95; 101; 99 Mean: 94% RSD: 7%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: - The column is a semi-capillary column DB-210 (15 m, 530 µm i.d., 1 µm film thickness) - Carrier gas: helium, 14.4 mL/min - Temperature programme: 90°C hold 1 min then 50°C/min to 220°C hold 3.55 min and 10°C/min to 240°C hold 10.85 min - Injection: 2 µL, 90°C hold 0.20 min then 180°C/min to 250°C hold 16.92 min
Conclusion	Agree with the applicant's version with above amendments.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.2	**Surface water**	
	1. REFERENCE	**Official use only**
1.1 **Reference**	**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 XXXX 7 October 1999 ; (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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 **Guideline study**	OPPTS 860.1340	
2.2 **GLP**	Yes	
2.3 **Deviations**	None	
	3. MATERIALS AND METHODS	
3.1 **Preliminary treatment**		
3.1.1 Enrichment	None	
3.1.2 Cleanup	Samples are filtered on a filter paper	**X**
3.2 **Detection**		
3.2.1 Separation method	LC-MS/MS	**X**
3.2.2 Detector	Mass Selective Detector MS/MS	**X**
3.2.3 Standard(s)	External standardisation	
3.2.4 Interfering substance(s)	None	
3.3 **Linearity**		
3.3.1 Calibration range	0.01 to 2µg/l	**X**
3.3.2 Number of measurements	6	**X**

Section A4.2.3.2		**Surface water**	
3.3.3	Linearity	Correlation coefficient fipronil r^2>0.999112 XXXX r^2>0.998569 XXXX r^2>0.999240 XXXX r^2>0.996952	
3.4	**Specifity: interfering substances**	None	
3.5	**Recovery rates at different levels**	For each compound and at each fortification level, the mean of recoveries was between 70% and 120% See Table A4.2.3.2.1-1	
3.5.1	Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% (with only one exception for XXXX at the limit of quantification which was slightly above 20%) See Table A4.2.3.2.1-1	
3.6	**Limit of determination**	Limit of determination is 0.01 µg/l for each analyte (detection limit: 0.004 µg/l)	
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1	
3.7.2	Independent laboratory validation	Not required	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Following the addition of acetonitrile (20%, v/v) to the surface water, residues were quantified through injection into a Liquid Chromatography (LC) using a Mass Selective Detector (MS/MS). For surface with high turbidity, the samples were filtered trough a filter paper. The sediment was then washed with acetonitrile. Fipronil and its metabolites (XXXX, XXXX and XXXX) were analysed by Liquid Chromatography (LC) using a Mass Selective Detector (MS/MS) and external standardisation.	
4.2	**Conclusion**	Specificity: Parent compound and its metabolites (XXXX) can be determined due to the detection technique. Repeatability: see results of RSD (Relative Standard Deviation) below Table A4.2.3.2.1-1 Limit of quantification (LOQ): Surface water: 10 ng/L for each product (Limit of Detection: 5 ng/L) Confirmatory method: Not required since use of a mass selective detector	**x**

Section A4.2.3.2	**Surface water**	
	Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification as well as several times this limit. The recovery results reported in the Table A4.2.3.2.1-1 are a compilation of results obtained with surface water sampled from 7 different localities. The recovery results from the method validation are presented in the following Table A4.2.3.2.1-1. **Conclusion:** The method referenced in this section was successfully validated for the surface water at 10.0 ng/L (except for XXXX whose % RSD is slightly above 20%), 100.0 ng/L and 2000.0 ng/L for Fipronil and each of its metabolites.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.2.3.2.1-1

	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n=7)	<30%LOQ (n=7)	<30%LOQ (n=7)	<30%LOQ (n=7)
10.0 ng/L	71.4; 61.1; 57.0 75.7; 85.0; 79.7 106.6; 64.6; 70.5 57.4; 52.2; 72.8 76.0; 63.7; 64.5 68.6; 71.3; 61.5 80.7; 94.6 Mean: 71.5% RSD: 18.4%	77.5; 72.4; 77.5 72.4; 91.2; 92.3 103.3; 84.9; 90.2 42.7; 40.0; 58.7 58.1; 72.5; 64.9 70.7; 76.4; 55.3 Mean: 72.2% RSD: 22.5%	86.1; 79.5; 91.7 76.5; 89.3; 92.5 103.9; 92.2 106.8; 62.0; 74.3 85.7; 83.0; 88.6 76.6; 89.8; 90.1 75.3; 83.7; 81.0 Mean: 85.4% RSD: 11.0%	84.0; 83.8; 79.0 78.0; 91.4; 98.5 97.7; 74.1; 75.5 51.9; 46.8; 78.0 67.3; 68.2; 68.8 72.3; 75.3; 66.9 98.3; 69.8 Mean: 76.3% RSD: 18.1%
100.0 ng/L	99.4; 92.6; 87.3 87.7; 86.6; 83.5 85.7; 85.7; 83.6 95.3; 91.5; 90.0 90.6; 96.0; 92.0 93.5 Mean: 89.4% RSD: 4.7%	100.8; 94.5; 88.1 89.6; 87.7; 88.8 89.6; 87.7; 88.8 94.9; 92.3; 91.7 89.2; 85.3; 86.6 89.4 Mean: 89.4% RSD: 2.8%	101.3; 91.6; 84.4 91.4; 90.0; 86.3 86.0; 87.3; 87.9 101.0; 96.0; 97.7 85.2; 84.2; 84.5; 85.9 Mean: 90.0% RSD: 6.6%	99.5; 94.1; 80.0 88.7; 86.0; 82.3 85.7; 83.4; 83.4 95.4; 95.7; 93.2 87.5; 87.8; 91.3 91.0 Mean: 88.6% RSD: 5.1%
2000.0 ng/L	92.8; 97.1; 111.2 66.6; 57.0; 98.7 100.0; 96.7; 94.6 95.2 Mean: 90.9% RSD: 17.9%	89.3; 96.9; 104.9 67.0; 57.8; 96.1 96.6; 94.5; 92.3 94.1 Mean: 88.9% RSD: 16.5%	91.8; 98.3; 96.6 67.8; 62.2; 95.8 93.0; 97.9; 91.7 92.8 Mean: 88.8% RSD: 14.5%	88.8; 92.8; 96.7 62.5; 54.3; 91.0 94.7; 91.0; 90.0 95.7 Mean: 85.8% RSD: 17.2%

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**3.1.2 Cleanup** *Samples* with high sediment content *are filtered on a filter paper*, followed by washing of the sediments on filter paper with acetonitrile. **3.2.1 Separation method** The details on the chromatographic method are missing: - The columns: two of the following, closely coupled columns: YMC ODS-AQ, 2.0×50 mm, 3 µm particle size, 120 A pore size. These two columns can be submitted with one 2.0×100 mm column of the same type - The mobile phase is composed of 68% Acetonitrile/ 32% (1.5% Acetic Acid in water) - The injection is 35 µL (can be increased) and the flow rate is 0.2250 mL/min **3.2. Detection method** Add the type of ionization: Electrospray (TurboIonSpray) – negative ion mode **3.3.1 Calibration range** 5-100 ng/l + validation of the linearity at 2000ng/l **3.3.2 Number of measurements** ~~6~~ >10 **4.2 conclusion** *(Limit of Detection: ~~5~~ 4 ng/L)*
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	Surface waters with high sediments need to be filtered on filter paper (followed by washing the sediments with acetonitrile), to get good recoveries at 10 ng/l. At 100 ng/l recoveries with non-filtered waters exceed 70%. For turbid waters, prefer a method based on liquid-liquid (A4.2.3.2/04, A4.2.3.2/05) .
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.2	**Surface water**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.3.2/02** Fuchsbichler, G. (1999) Method validation study for Fipronil and its metabolites (XXXX) in Surface water (river, Pond) XXXX 15 January 1999; (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Extraction and clean-up using a fipronil immuno affinity cartridge	
3.1.2	Cleanup	See above	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography	**X**
3.2.2	Detector	Electron Capture	
3.2.3	Standard(s)	External standardisation	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.002 to 0.02 µg/ml	
3.3.2	Number of measurements	5	**X**

Section A4.2.3.2	**Surface water**	
3.3.3 Linearity	coefficient fipronil r^2>0.9875 XXXX r^2>0.9899 XXXX r^2>0.9866 XXXX r^2>0.9887	
3.4 Specifity: interfering substances	None	
3.5 Recovery rates at different levels	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% Tables A4.2.3.2.2-1 to A4.2.3.2.2-3	
3.5.1 Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% Tables A4.2.3.2.2-1 to A4.2.3.2.2-3	
3.6 Limit of determination	Limit of determination 0.2 µg/l for each compound.	
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Residues in surface water were extracted from the samples using a Fipronil immuno-affinity column. Residues were quantified by Gas Chromatography (GC) on a semi-capillary column using an Electron Capture Detector (ECD) and external standardisation.	
4.2 Conclusion	Specificity: Fipronil and its metabolites (XXXX) can be determined. The specificity of the method was assured by the sample preparation (use of a Fipronil immuno-affinity column). Repeatability: See results of RSD (Relative Standard Deviation) below Tables A4.2.3.2.2-1 to A4.2.3.2.2-3 Limit of Quantification (LOQ): 0.2 µg/L for each product Confirmatory method: None Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification as well as ten times this limit. Results from the method validation are presented in the following Tables A4.2.3.2.2-1 to A4.2.3.2.2-3 **Conclusion:** The method referenced in this section was successfully validated for the following surface water substrates: - River and pond waters at 0.2 and 2.0 µg/L for Fipronil and each of its metabolites	

Section A4.2.3.2	**Surface water**	

4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.2.3.2.2-1

River water				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)
0.2 µg/L	95; 100; 89 94; 95 Mean: 95% RSD: 4%	100; 95; 90; 80; 82 Mean: 89% RSD: 9%	90; 90; 80; 90; 100 Mean: 90% RSD: 7%	92; 96; 87 96; 92 Mean: 93% RSD: 4%
2.0 µg/L	96; 100; 100; 100; 108 Mean: 101% RSD: 4%	100; 94; 100; 92; 96 Mean: 96% RSD: 3%	91; 96; 100; 100; 104 Mean: 98% RSD: 5%	104; 100; 97; 100; 109 Mean: 102% RSD: 4%

Table A4.2.3.2.2-2

Pond water 1				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 3)	<30%LOQ (n = 3)	<30%LOQ (n = 3)	<30%LOQ (n = 3)
0.2 µg/L	83; 85; 87; 100; 75 Mean: 86% RSD: 9%	82; 82; 92; 108; 80 Mean: 89% RSD: 12%	100; 89; 79; 100; 81 Mean: 90% RSD: 10%	87; 93; 81; 100; 84 Mean: 89% RSD: 8%
2.0 µg/L	100; 85; 105; 100; 87 Mean: 95% RSD: 8%	91; 71; 83; 95; 88 Mean: 86% RSD: 10%	95; 84; 105; 94; 81 Mean: 92% RSD: 9%	88; 78; 100; 100; 74 Mean: 88% RSD: 12%

Table A4.2.3.2.2-3

Pond water 2				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)
0.2 µg/L	100; 81; 77; 95; 83 Mean: 87% RSD: 10%	91; 86; 71; 93; 85 Mean: 85% RSD: 9%	97; 74; 88; 91; 108 Mean: 92% RSD: 12%	88; 77; 90; 104; 107 Mean: 93% RSD: 12%
2.0 µg/L	100; 98; 89; 100; 98 Mean: 97% RSD: 4%	95; 102; 82; 87; 106 Mean: 96% RSD: 9%	95; 100; 93; 107; 104 Mean: 100% RSD: 5%	102; 98; 98; 104; 102 Mean: 101% RSD: 2%

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: - The column is DB-5 (15 m, 0.25 mm i.d., 0.25 µm film thickness) - Carrier gas: helium 4.6, 3.0 ml/min - Temperature programme: 100°C, 1 min then 15°C/min to 180°C, 1 min and 6°C/min to 250°C, 10 min - Injection: 1 µL, 250°C **3.3.2 Number of measurements** 8
Conclusion	Accept applicant's version with above amendment
Reliability	2
Acceptability	Acceptable
Remarks	In spite of coefficients of linearity slightly below 0.99 for each compound, the recovery rates (mean = 94%) and RSD (<12%) are good. Thus the method is fully validated. Whenever possible LC-MS/MS should be used instead of GC-ECD.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.2	**Surface water**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.3.2/03** Lopes, A. (1997) Validation of method of analysis for the determination of Fipronil and its metabolites in water XXXX 19 November 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not applicable	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Yes : using C18-ENV Sep-Pak Plus cartridge	**X**
3.1.2	Cleanup		
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography	**X**
3.2.2	Detector	Electron Capture	
3.2.3	Standard(s)	External standardisation	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	1 µg/l to 10 µg/l	**X**
3.3.2	Number of measurements	5	

Section A4.2.3.2	**Surface water**	
3.3.3 Linearity	coefficient fipronil r^2>0.9966 XXXX r^2>0.9991 XXXX r^2>0.9952 XXXX r^2>0.9691 XXXX r^2>0.9894	**X**
3.4 Specifity: interfering substances	None	
3.5 Recovery rates at different levels	For each compound and at each fortification level, the mean of recoveries was between 70% and 110% Tables A4.2.3.2.3-1 to A4.2.3.2.3-3	**X**
3.5.1 Relative standard deviation	For each compound and at each fortification level, the mean RSD was lower than 20% Tables A4.2.3.2.3-1 to A4.2.3.2.3-3	**X**
3.6 Limit of determination	Limit of determination 1 µg/l for each compound.	
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Residues of Fipronil and its metabolites (XXXX) were extracted from the water samples (surface, ground, rice paddy) with toluene. A trace enrichment of the XXXX was performed by passing the aqueous phase through a C18-ENV Sep-Pak Plus cartridge. The methanol eluate was evaporated to dryness and then hydrolysed at 70°C to XXXX with sulphuric acid in acetonitrile/methanol. After neutralisation, evaporation of the acetonitrile and addition of water; the residues were partitioned into toluene. The toluene phase containing Fipronil and its metabolites was analysed by Gas Chromatography (GC) using an Electron Capture Detector (ECD) and external standardisation.	
4.2 Conclusion	Specificity: Fipronil and its metabolites (XXXX) can be determined due to the detection technique The metabolite XXXX can be quantified following an acidic hydrolysis into the compound XXXX. Repeatability: See results of RSD (Relative Standard Deviation) below Tables A4.2.3.2.3-1 to A4.2.3.2.3-3 Limit of quantification (LOQ): Surface water, ground water, rice paddy water: 1 µg/L for each product.	**X**

Section A4.2.3.2	**Surface water**	
	Confirmatory method: Yes, GC-MSD Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification as well as several times this limit. Results from the method validation are presented in the following Tables A4.2.3.2.3-1 to A4.2.3.2.3-3. **Conclusion**: The method referenced in this section was successfully validated for the following water substrates: - Surface water at 1.0 and 10.0 µg/L for Fipronil and each of its metabolite - Ground water at 1.0 and 10.0 µg/L for Fipronil and each of its metabolite - Rice paddy water at 1.0, 10.0 and 50.0 µg/L for Fipronil and each of its metabolite (except for XXXX at 10.0 µg/L whose % RSD is >20%)	**x**
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.2.3.2.3-1

Surface water						
	Fipronil	**XXXX**	**XXXX**	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)
1.0 µg/L	98.3; 99.1; 98.1 104.5; 106.4 Mean: 101.3% RSD: 3.8%	101.1; 98.4; 95.0 104.7; 105.8 Mean: 101.1% RSD: 4.4%	96.7; 98.7; 98.3 94.7; 97.9 Mean: 97.3% RSD: 1.7%	97.2; 98.1 96.4 92.7; 97.3 Mean: 96.3% RSD: 2.2%	102;8; 99.7; 98.4 111.6; 110.9 Mean: 104.7% RSD: 5.9%	89;4; 96;4 94.3 95.3; 97;0 Mean: 94.5% RSD: 3.2%
10.0 µg/L	92.6; 83.3; 95.5 77.8; 82.5 Mean: 86.3% RSD: 8.6%	94.9; 84.1; 102.3 80.0; 89.0 Mean: 90.1% RSD: 9.8%	103.1; 96.7; 103.3 85.7; 97.4 Mean: 97.2% RSD: 7.4%	94.7; 84.2; 88;6 74.4; 79.2 Mean: 84.2% RSD: 9.4%	85.5; 74.7; 92.7 73.2; 76.4 Mean: 80.5% RSD: 10.3%	96;6; 92.1; 96.5 95.7; 94.4 Mean: 95.1% RSD: 2.0%

Table A4.2.3.2.3-2

Ground water						
	Fipronil	**XXXX**	**XXXX**	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)
1.0 µg/L	113.2; 119.1; 115.2 119.6; 114.7 Mean: 116.4% RSD: 2.4%	116.5; 116.1; 120.8 128.2; 118.8 Mean: 120.1% RSD: 4.1%	96.9; 98.0; 98.5 101.2; 99.2 Mean: 98.8% RSD: 1.6%	112.2; 109.1; 112.3 121.8; 112.1 Mean: 113.5% RSD: 4.3%	127.2; 131.7; 127.1 149.2; 136.5 Mean: 134.3% RSD: 6.8%	97.7; 90.5; 92.3 93.4; 93.9 Mean: 93.6% RSD: 2.8%
10.0 µg/L	98.0; 95.5; 97.6 98.7; 95.8 Mean: 97.7% RSD: 1.4%	96.6; 95.7; 96.8 108.2; 98.6 Mean: 99.2% RSD: 5.2%	97.7; 95.6; 95.4 97.2; 98.7 Mean: 96.9% RSD: 1.5%	98.0; 93.2; 105.1 98.3; 100.8 Mean: 99.1% RSD: 4.4%	90.6; 91.1; 95.2 91.9; 99.8 Mean: 93.7% RSD: 4.1%	92.1; 91.6; 92.5 89.2; 93.2 Mean: 91.7% RSD: 1.7%

Table A4.2.3.2.3-3

Rice paddy water						
	Fipronil	**XXXX**	**XXXX**	**XXXX**	**XXXX**	**XXXX**
Control value	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)	<30%LOQ (n = 2)
1.0 µg/L	91.6; 98.0; 92.6 106.1; 102.0 Mean: 98.1% RSD: 6.3%	90.6; 93.2; 87.7 116.6; 105.2 Mean: 98.7% RSD: 12.2%	80.6; 88.0 83.2 94.3; 91.6 Mean: 87.5% RSD: 6.5%	86.9; 95.8; 86.0 104.3; 98.4 Mean: 94.3% RSD: 8.3%	109.9; 107.8; 105.0 109.8; 110.4 Mean: 108.6% RSD: 2.1%	95.4; 96.1; 101.1 90.5; 71.2 Mean: 90.9% RSD: 12.8%
10.0 µg/L	76.8; 67.8; 104.7 106.1; 100.0 Mean: 91.1% RSD: 19.3%	77.3; 67.9; 107.1 106.6; 109.5 Mean: 93.7% RSD: 20.9%	85.6; 79.2; 99.4 98.8; 97.9 Mean: 92.2% RSD: 10.0%	75;6; 64.8; 102.1 102.6; 97.2 Mean: 88.5% RSD: 19.5%	73.4; 61.5; 110.6 109.7; 91.3 Mean: 89.3% RSD: 24.4%	98.9; 95;7; 90;8 78.1; 84.2 Mean: 89.5% RSD: 9.5%
50.0 µg/L	105.6; 119.2; 17.4 106.6; 107.0 Mean: 111.2% RSD: 5.9%	93.4; 114.8; 110.2 99.2; 98.8 Mean: 103.3% RSD: 8.6%	100.6; 102.2; 103.6 101.6; 100.8 Mean: 101.8% RSD: 1.2%	105.0; 114.4; 113.6 105.0; 103.2 Mean: 108.2% RSD: 4.9%	91.2; 127.0; 119.0 96.4; 94.8 Mean: 105.7% RSD: 15.3%	93.6; 90.4; 90.6 95.0; 95.0 Mean: 92.9% RSD: 2.5%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**3.1.1 Enrichment** ~~*Yes : using C18-ENV Sep-Pak Plus cartridge*~~ Residues of Fipronil and its metabolites (XXXX) were extracted from the water samples (surface, ground, rice paddy) with toluene. For XXXX only : passing through a C18-ENV Sep-Pak Plus cartridge and then hydrolysing at 70°C to XXXX with sulphuric acid in acetonitrile/methanol followed by neutralisation, evaporation of the acetonitrile, addition of water, and finally partitioning into toluene. **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is J&W Scientific DB1701 (15 m, 0.32 mm i.d., 0.25 µm film thickness) - Carrier gas: helium, 2-3 mL/min - Temperature programme: 50°C, 1 min then ramp 30°C/min to 200°C, hold 20 min, ramp 30°C/min to 230°C, hold 10 min and ramp 30°C/min to 250°C, hold 12 min - Injection: 0.5 µL, 280°C **3.3.3 Calibration range** *1 µg/l to ~~10~~ 8 µg/l* **3.3.4 Linearity** Add: XXXX r^2>0.9985 **3.5 Recovery rates at different levels** ~~*For each compound and at each fortification level, the mean of recoveries was between 70% and 110%*~~ For each compound and at each fortification level, the mean of recoveries in surface water and rice paddy water was between 80% and 110%. In ground water, the means of recoveries were between 92% and 134%. **3.5.1 Relative standard deviation** ~~*For each compound and at each fortification level, the mean RSD was lower than 20%*~~ For each compound and at each fortification level, the mean RSD was lower than 10% for surface water and ground water. For rice paddy water, 4 of 6 mean RSD at 10 µg/l are between 19% and 24% **4.2 Conclusion** *The method referenced in this section was successfully validated for the following water substrates:* *- Surface water at 1.0 and 10.0 µg/L for Fipronil and each of its metabolite* ~~*- Ground water at 1.0 and 10.0 µg/L for Fipronil and each of its metabolite*~~ ~~*- Rice paddy water at 1.0, 10.0 and 50.0 µg/L for Fipronil and each of its metabolite (except for XXXX at 10.0 µg/L whose % RSD is >20%)*~~

	The method was not validated for ground water and rice paddy water for Fipronil and each of its metabolite.
Conclusion	The method was validated only for surface water, but not for ground water and rice paddy water. Furthermore, the detection of XXXX is not satisfactory (r^2>0.97%). Adopt applicant's version with above amendment, only for surface water, and with reservation for XXXX.
Reliability	2
Acceptability	Acceptable only for surface water, and with reserves for XXXX.
Remarks	For the analysis of Fipronil and its metabolites in surface water, prefer the other methods presented in this section. Whenever possible LC-MS/MS should be used instead of GC-ECD.

	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.2	**Drinking water / Surface water**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**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 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 91/414; SANCO/3029/99 rev. 4 (11 July 2000) SANCO/825/00 rev. 7 (17 March 2004); EPA 850.7100	**X**
2.2	**GLP**	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany Fed.Rep.)	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	An aliquot of the water sample is adjusted to about pH 2 using 50 µL of concentrated formic acid. After adding 5.0 mL of DCM the sample is shaken on a mechanical shaker and centrifuged. After phase separation a 60% aliquot of the DCM phase is evaporated to dryness. The residues are redissolved in water/methanol (30 + 70, v + v) and measured using LC-MS/MS.	**X**
3.1.2	Cleanup	None	
3.2	**Detection**		
3.2.1	Separation method	LC-MS/MS	**X**
3.2.2	Detector	Mass spectrometric detector	**X**
3.2.3	Standard(s)	External standardisation	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	5 concentrations were used	

Section A4.2.3.2	**Drinking water / Surface water**	
3.3.2 Number of measurements	2	
3.3.3 Linearity	Correlation coefficient Fipronil $r^2 = 0.9984$ XXXX $r^2 = 0.9994$ XXXX $r^2 = 0.9992$ XXXX $r^2 = 0.9996$	
3.4 Specificity: interfering substances	The method allows the specific determination of BAS 350 I (Fipronil) and its metabolites XXXX, XXXX and XXXX in water.	x
3.5 Recovery rates at different levels	Drinking and and surface water samples were spiked with Fipronil, XXXX, XXXX and XXXX at the limit of quantification (LOQ) and at 10 x LOQ. The recovery data are corrected for interferences of the appropriate unfortified sample. Results from the method validation are presented in Tables A4.2.3.2.4-1 and A4.2.3.2.4-2 (corrected recoveries for first mass transition).	
3.5.1 Relative standard deviation	Results are shown in Tables A4.2.3.2.4-1 and A4.2.3.2.4-2.	
3.6 Limit of determination	Drinking and surface water: 0.004 µg/kg for each analyte.	
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	The analytical method is used to determine residues of the active substance Fipronil and its metabolites (XXXX, XXXX, XXXX) in water by means of LC-MS/MS after extraction of water samples (drinking and surface water) with DCM. Quantification is carried out by external standardisation).	
4.2 Conclusion	The method referenced in this section was successfully validated according to the European requirements as defined in document 96/46/EC of 16 July 1996 for the following water substrates: - Drinking water at 0.004 and 0.04 µg/kg for Fipronil and each of its metabolite - Surface water at 0.004 and 0.04 µg/kg for Fipronil and each of its metabolite With one exception the tested untreated water samples showed no significant interferences at the retention times of Fipronil, XXXX, XXXX and XXXX. The blank for XXXX in surface water was ~ 50% of the LOQ, which exceeds the limit set by the European guideline (30% LOQ). All other blank values in both tap and surface water were < 13% LOQ.	
4.2.1 Reliability	1	

Section A4.2.3.2	**Drinking water / Surface water**	

4.2.2 Deficiencies	None	

Table A4.2.3.2.4-1

Drinking water				
	Fipronil	XXXX	XXXX	XXXX
Control	0.0000 µg/kg <30%LOQ (n = 2)	0.0000 µg/kg <30%LOQ (n = 2)	0.0003 µg/kg <30%LOQ (n = 2)	0.0002 µg/kg <30%LOQ (n = 2)
0.004 µg/kg	91.0; 90.4; 91.4 85.8; 92.0 Mean: 90.1% RSD: 2.8%	91.8; 97.5; 100.9 100.1; 103.8 Mean: 98.8% RSD: 4.6%	99.9; 98.7; 86.7 86.0; 87.1 Mean: 91.7% RSD: 7.6%	99.7; 101.8 98.5 96.2; 99.1 Mean: 99.1% RSD: 2.0%
0.04 µg/kg	86.3; 86.5; 64.9 111.8; 87.4 Mean: 87.4% RSD: 19.0%	107.5; 94.3; 81.8 97.8; 110.0 Mean: 98.3% RSD: 11.5%	96.3; 99.4; 87.5 105.1; 101.2 Mean: 97.9% RSD: 6.8%	100.7; 99.2; 90.6 108.2; 97.7 Mean: 99.3% RSD: 6.4%

Table A4.2.3.2.4-2

Surface water				
	Fipronil	XXXX	XXXX	XXXX
Control	0.0004 µg/kg <30%LOQ (n = 2)	0.0003 µg/kg <30%LOQ (n = 2)	0.0005 µg/kg <30%LOQ (n = 2)	0.0020 µg/kg 50%LOQ (n = 2)
0.004 µg/kg	105.3; 100.9; 97.9 87.8; 98.4 Mean: 98.1% RSD: 6.6%	89.2; 104.6; 101.1 97.0; 98.9 Mean: 98.1% RSD: 5.9%	94.9; 91.5; 91.5 91.5; 88.8 Mean: 91.6% RSD: 2.4%	97.9; 90.4 102.8 95.5; 93.3 Mean: 96.0% RSD: 4.9%
0.04 µg/kg	96.1; 94.8; 87.6 91.2; 92.7 Mean: 92.5% RSD: 3.6%	109.0; 106.6; 103.5 109.6; 106.5 Mean: 107.0% RSD: 2.3%	97.5; 96.9; 94.2 106.3; 93.3 Mean: 97.6% RSD: 5.3%	97.4; 95.3; 91.8 96.5; 100.0 Mean: 96.2% RSD: 3.1%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**2.1Guideline study** ~~*EEC 91/414*~~ 96/46/EC of 16 July 1996 **3.1.1 Enrichment** Explicit the nature and characteristics of DCM (not given in the report) **3.2.1 Separation method** The details on the chromatographic method are missing: - The column: Betasil C18, 100×2.1 mm ID, 5 µm - Mobile phase: solvent A is composed of water + 0.1% formic acid and the solvent B is composed of methanol + 0.1% formic acid - The injection is 10 µL and the flow rate is 0.6 mL/min **3.2. Detection method** Add the type of ionization: negative ion mode **3.4 Specificity: interfering substances** add The blank for XXXX in surface water was ~ 50% of the LOQ, which exceeds the limit set by the European guideline (30% LOQ). The recovery value were corrected for this interference.
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.3.2	Surface water	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**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 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 91/414; EEC 96/46; SANCO/825/00 rev. 7 (17 March 2004); SANCO/3029/99 rev. 4 (11 July 2000); EPA 850.7100	x
2.2	**GLP**	Yes (laboratory certified by Landesamt fuer Umweltschutz und Gewerbeaufsicht, Mainz, Germany)	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	An aliquot of the water sample is adjusted to about pH 2 using 50 µL of concentrated formic acid. After adding 5.0 mL of DCM the sample is shaken on a mechanical shaker and centrifuged. After phase separation a 60% aliquot of the DCM phase is evaporated to dryness. The residues are redissolved in water/methanol (30 + 70, v + v) and measured using LC-MS/MS.	x
3.1.2	Cleanup	None	
3.2	**Detection**		
3.2.1	Separation method	LC-MS/MS	x
3.2.2	Detector	Mass spectrometric detector	x
3.2.3	Standard(s)	External standardisation	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	5 concentrations	

Section A4.2.3.2	**Surface water**	
3.3.2 Number of measurements	1	
3.3.3 Linearity	Correlation coefficient Fipronil $r^2 = 0.9999$ XXXX $r^2 = 0.9997$ XXXX $r^2 = 0.9998$ XXXX $r^2 = 0.9997$ XXXX $r^2 = 0.9998$	
3.4 Specificity: interfering substances	The method allows the specific determination of BAS 350 I (Fipronil) and its metabolites XXXX, in water.	
3.5 Recovery rates at different levels	Drinking and surface water samples were spiked with Fipronil, XXXX at the limit of quantification (LOQ), at 10 x LOQ and 100 x LOQ. The recovery data are corrected for interferences of the appropriate unfortified sample. Results from the method validation are presented in Tables A4.2.3.2.5-1 and A4.2.3.2.5-2 (corrected recoveries for first mass transition).	
3.5.1 Relative standard deviation	Tables A4.2.3.2.5-1 and A4.2.3.2.5-2	
3.6 Limit of determination	Drinking and surface water: 0.01 µg/kg for each analyte	
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	The analytical method is used to determine residues of the active substance Fipronil and its metabolites (XXXX in water by means of LC-MS/MS after extraction of water samples (drinking and surface water) with DCM. Quantification is carried out by external standardisation).	
4.2 Conclusion	The method referenced in this section was successfully validated according to the European requirements as defined in document 96/46/EC of 16 July 1996 for the following water substrates: - Drinking water at 0.01, 0.10 and 1.0 µg/kg for Fipronil and each of its metabolite - Surface water at 0.01, 0.10 and 1.0 µg/kg for Fipronil and each of its metabolite The tested untreated water samples showed no significant interferences at the retention times of Fipronil, XXXX. All of the control samples analysed were below the limit of 30% LOQ set by the European guidance document on residue analytical methods SANCO/825/00 rev. 7.	
4.2.1 Reliability	1	

Section A4.2.3.2	**Surface water**	
4.2.2 Deficiencies	None	

Table A4.2.3.2.5-1

Drinking water					
	Fipronil	XXXX	XXXX	XXXX	XXXX
Control	0.0003 µg/kg <30%LOQ (n = 3)	0.0005 µg/kg <30%LOQ (n = 3)	0.0001 µg/kg <30%LOQ (n = 3)	0.0000 µg/kg <30%LOQ (n = 3)	0.0001 µg/kg <30%LOQ (n = 3)
0.010 µg/kg	107.7; 98.0; 96.8 99.0; 93.0 Mean: 98.9% RSD: 5.5%	98.5; 98.0; 91.9 98.7; 89.9 Mean: 95.4% RSD: 4.4%	103.2; 106.6; 103.0 101.6; 100.5 Mean: 103.0% RSD: 2.2%	99.8; 105.7 85.6 90.8; 90.7 Mean: 94.5% RSD: 8.5%	111.7; 102.6 80.6 93.1; 90.9 Mean: 95.8% RSD: 12.4%
0.10 µg/kg	98.0; 100.2; 96.6 103.3; 96.5 Mean: 98.9% RSD: 2.9%	96.7; 103.9; 98.4 98.3; 99.4 Mean: 99.3% RSD: 2.8%	102.1; 99.9; 107.8 102.1; 101.0 Mean: 102.6% RSD: 3.0%	92.8; 94.1 96.8 98.0; 93.5 Mean: 95.0% RSD: 2.4%	91.7; 93.9 103.4 103.0; 104.8 Mean: 99.3% RSD: 6.1%
1.00 µg/kg	98.8; 103.5; 101.9 107.2; 107.3 Mean: 103.7% RSD: 3.5%	98.1; 97.1; 104.4 97.3; 106.9 Mean: 100.8% RSD: 4.5%	106.4; 103.8; 106.4 97.7; 104.6 Mean: 103.8% RSD: 3.4%	98.4; 101.5; 94.4 99.5; 105.8 Mean: 99.9% RSD: 4.2%	101.1; 98.6; 104.1 105.7; 99.7 Mean: 101.9% RSD: 2.9%

Table A4.2.3.2.5-2

Surface water					
	Fipronil	XXXX	XXXX	XXXX	XXXX
Control	0.0008 µg/kg <30%LOQ (n = 3)	0.0003 µg/kg <30%LOQ (n = 3)	0.0002 µg/kg <30%LOQ (n = 3)	0.0020 µg/kg <30%LOQ (n = 3)	0.0001 µg/kg <30%LOQ (n = 3)
0.010 µg/kg	108.1; 104.9; 102.8 97.4; 101.2 Mean: 102.9% RSD: 3.9%	102.7; 104.9; 97.5 102.8; 100.2 Mean: 101.6% RSD: 2.8%	100.6; 103.8; 105.1 102.7; 101.2 Mean: 102.7% RSD: 1.8%	98.2; 101.5 100.5 98.9; 95.8 Mean: 99.0% RSD: 2.2%	101.8; 106.6 108.0 108.5; 108.2 Mean: 106.6% RSD: 2.6%
0.10 µg/kg	98.2; 104.3; 106.9 105.5; 100.7 Mean: 103.1% RSD: 3.5%	103.5; 106.2; 102.0 102.2; 96.5 Mean: 102.1% RSD: 3.5%	102.7; 99.3; 97.6 102.9; 96.2 Mean: 99.7% RSD: 3.0%	101.2; 93.1 102.7 104.7; 105.3 Mean: 101.4% RSD: 4.8%	96.4; 98.7 100.4 107.0; 93.3 Mean: 99.2% RSD: 5.2%
1.00 µg/kg	105.5; 97.2; 99.2 101.7; 104.6 Mean: 101.7% RSD: 3.4%	96.9; 104.9; 101.5 101.3; 103.5 Mean: 101.6% RSD: 3.0%	102.5; 100.8; 97.6 100.4; 103.6 Mean: 101.0% RSD: 2.3%	97.9; 99.6; 102.1 100.1; 103.8 Mean: 100.7% RSD: 2.3%	98.3; 100.6; 91.9 104.6; 106.2 Mean: 100.3% RSD: 5.6%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised January 2010
Materials and methods	**2.1Guideline study** ~~*EEC 91/414*~~ 96/46/EC of 16 July 1996 **3.1.1 Enrichment** The nature and characteristics of DCM (not given in the report) should have been explicited. It is assumed that DCM stands for Dichloromethane. **3.2.1 Separation method** The details on the chromatographic method are missing: - The column: Betasil C18, 100×2.1 mm ID, 5 µm - Mobile phase: solvent A is composed of water + 0.1% formic acid and the solvent B is composed of methanol + 0.1% formic acid - The injection is 10 µL and the flow rate is 0.6 mL/min **3.2. Detection method** Add the type of ionization: negative ion mode
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4	**Animal and human body fluids and tissues**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A4.2.4/01** Beaudonnet, J.-P. (1998) Fiproles determination in blood plasmas XXXX 31 March 1998; (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	
2.2	**GLP**	Yes	**X**
2.3	**Deviations**	Not relevant	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Extracted over a Solid phase extraction (C18) cartridge	
3.1.2	Cleanup	Toluene liquid – liquid extraction	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC)	**X**
3.2.2	Detector	Electron capture	
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	The linearity of the method was verified in the range 2 to 100 µg/l for each product	
3.3.2	Number of measurements	4	
3.3.3	Linearity	coefficient fipronil r^2>1.000 XXXX r^2>0.999 XXXX r^2>1.000	

Section A4.2.4		Animal and human body fluids and tissues	
3.4	**Specifity: interfering substances**	None	
3.5	**Recovery rates at different levels**	fipronil 87 – 98% : Mean 91% XXXX 76 – 99% : Mean 89% XXXX 88 – 100% : Mean 95%	x
3.5.1	Relative standard deviation	fipronil 4.3% XXXX 9.7% XXXX 4.6%	
3.6	**Limit of determination**	Limit of quantification 1 µg/l for each compound	x
3.7	**Precision**		
3.7.1	Repeatability	RSD fipronil : 1.8 XXXX : 1.2 XXXX : 2.1	x
3.7.2	Independent laboratory validation	Not required	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Residues in human plasma samples were extracted over a C18 cartridge using a methanol/water (80/20, v/v) elution mixture. Sample clean up was performed using a toluene liquid – liquid extraction	x
4.2	**Conclusion**	Specificity: parent compound and its metabolites (XXXX). Repeatability: fipronil :recovery 87 – 98% (mean 92.5%) RSD 4.31% XXXX :recovery 76 – 99% (mean 89.3%) RSD 9.67% XXXX :recovery 88 – 100% (mean 92.5%) RSD 4.57% limit of quantification 1.0 ng/ml Validation : the assay was validated by preparing and analysing spiked human plasma	
4.2.1	Reliability	1	x
4.2.2	Deficiencies	None	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised April 2010
Materials and methods	**2.2 GLP** ~~*Yes*~~ No **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is JW DB 210 (15 m, 0.53 mm, 1 µm film thickness) - Carrier gas: argon/methane (95/5), 11 mL/min - Temperature programme: 200°C, 20 min then 5°C/min to 230°C, 8 min, ramp 30°C/min to 230°C, hold 10 min and ramp 30°C/min to 250°C, hold 12 min - Injection: 3 µL, 220°C **3.2 Linearity** Number of measurements : 5 **3.5 Recovery rates at different levels** Only one level: about 50 µg/l for each analyte. **3.6 Limit of determination** The LOQ is the lowest level for which recovery was determined. *Limit of quantification ~~:~~50 µg/l for each compound* **3.7.1 Repeatability** *RSD* (%) after 6 measures of calibration solutions (2 µg/l of reference standards in methanol)
Conclusion	Adopt applicant's version with above amendments
Reliability	2 (not GLP, only one fortification level)
Acceptability	Acceptable
Remarks	The other method (A4.2.4/02) should be preferred as far as possible.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4	**Animal and human body fluids and tissues**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**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 XXXX GLP 19 February 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Purification over a C18 cartridge using a methanol/ultra pure water mixture for elution.	
3.1.2	Cleanup	Liquid–liquid extraction using toluene	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC)	**X**
3.2.2	Detector	Electron capture	
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	Fipronil, XXXX: 1.0, 20, 50, 100, 200, 300, 400 and 500 ng/mL XXXX: 0.70, 1.0, 2.5, 5.0, 7.5, 10, 12.5 and 15 ng/mL	
3.3.2	Number of measurements	5 different series were checked at each plasma concentrations:	

Section A4.2.4	**Animal and human body fluids and tissues**	
3.3.3 Linearity	Correlation coefficient fipronil r^2=0.9957 XXXX r^2=0.9954 XXXX r^2=0.9930 XXXX r^2=0.9928	
3.4 Specificity: interfering substances	No interferences	
3.5 Recovery rates at different levels	Human plasma samples were spiked with Fipronil, XXXX at the following concentrations: 50, 200 and 400 ng/mL for Fipronil, XXXX and 2.0, 5.0 and 8.0 ng/mL for XXXX Each concentration was measured six times. fipronil 94 – 99% XXXX 93 – 108% XXXX 91 – 102% XXXX 96 – 103%	
3.5.1 Relative standard deviation	fipronil 4.5 – 7.6% XXXX 3.7 – 8.6% XXXX 4.5 – 6.8% XXXX 4.0 – 12%	
3.6 Limit of determination	1.0 ng/mL for each compound	x
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	The analytical method is used to determine residues of the active substance Fipronil and its metabolites (XXXX) in human plasma by means of GC-ECD after purification of human plasma samples over a C18 cartridge using a methanol/water elution mixture followed by a liquid–liquid extraction using toluene. Quantification is carried out by external standardisation.	
4.2 Conclusion	The results obtained during the validation are within the limit of acceptance (± 20% for precision and 80-120% for accuracy). Consequently, the method can be used for the analyses of Fipronil and its metabolites (XXXX) contained in plasma samples in the studied range.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007, revised April 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: - The column is DB5 MS (30 m, 0.25 mm d., 0.25 µm film thickness) - Carrier gas: Nitrogen EL, 1.5 mL/min - Temperature programme: the initial temperature is 90°C during 0.5 min, the temperature rate is 40°C/min and the final temperature is 250°C during 5.5 min - Injection programme: 2 µL, the initial temperature is 90°C, the temperature rate is 250°C/min and the final temperature is 250°C during 8 min **3.6 Limit of determination** The LOQ is the lowest level for which recovery was determined. fipronil 50µg/L XXXX 50µg/L XXXX 50µg/L XXXX 2 µg/L
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4	**Animal and human body fluids and tissues**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.4/03** Pontal P.G. (1995) Validation of the method for the assay of Fipronil and its metabolites (XXXX) in human plasma XXXX GLP 28 June 1995 (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Purification over a C18 cartridge using a methanol/deionised water mixture for elution.	
3.1.2	Cleanup	Liquid extraction using toluene	
3.2	**Detection**		
3.2.1	Separation method	Gas-liquid Chromatography (GC)	**X**
3.2.2	Detector	Electron capture	
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	In some cases chromatographic interferences; see 3.4	
3.3	**Linearity**		
3.3.1	Calibration range	A series was checked at the following plasma concentrations: Fipronil, XXXX: 1.0, 2.5, 5.0, 7.5, 10 and 15 ng/mL	
3.3.2	Number of measurements	5	

Section A4.2.4	Animal and human body fluids and tissues	
3.3.3 Linearity	Correlation coefficient fipronil r^2=0.9964±0.00031 XXXX r^2=0.9936±0.00063 XXXX r^2=0.9968±0.00076	
3.4 Specificity: interfering substances	In some cases chromatographic interferences; then a second GC analysis was performed using a DB210 column to measure specifically the Fipronil level	
3.5 Recovery rates at different levels	Human plasma samples were spiked with Fipronil, XXXX at the following concentrations: 1.5, 5.0 and 10 ng/mL for Fipronil, XXXX Each concentration was measured nine times. fipronil 73 - 93 % XXXX 77 - 94 % XXXX 76 - 113 %	
3.5.1 Relative standard deviation	fipronil 2.3 - 4.6 % XXXX 5.4 - 6.9 % XXXX 3.7 - 11 %	
3.6 Limit of determination	1.0 ng/mL for each compound	x
3.7 Precision		
3.7.1 Repeatability	See 3.5.1	
3.7.2 Independent laboratory validation	Not required	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	The analytical method is used to determine residues of the active substance Fipronil and its metabolites (XXXX) in human plasma by means of GC-ECD after purification of human plasma samples over a C18 cartridge using a methanol/water elution mixture followed by a liquid extraction using toluene. Quantification is carried out by external standardisation.	
4.2 Conclusion	The results obtained during the validation are within the limit of acceptance (± 20% for precision and 80-120% for accuracy) for precision (2.3-11%), but not for accuracy (73-113%). However, recoveries are not much lower than 80%. Therefore, the method can be used for the analysis of Fipronil and its metabolites (XXXX) contained in plasma samples in the studied range.	x
4.2.1 Reliability	1	
4.2.2 Deficiencies	The accuracy of the method is not within the limit of acceptance.	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: The column is DB5, 15 m, 0.5 mm i.d., 1.5 µm film thickness - Carrier gas: Nitrogen C , 14 mL/min - Temperature column: 200°C - Injection: 220°C **3.6 Limit of determination** ~~1.0 ng/mL for each compound~~ This is not the LOQ. This is the limit of detection. For Fipronil, XXXX, LOQ=1.5 ng/mL **4.2 Conclusion** According to TNG on data requirements, the limit of acceptance for accuracy is ~~80-120%~~ 70-110%.
Conclusion	Different chromatographic stationary or mobile phases of different selectivity are acceptable confirmatory techniques. The validated method A4.2.4/02 uses a DB5 MS column (non polar), this method A4.2.4/03 uses a DB5 column (non polar too). Mobile phases are nitrogen for the method A4.2.4/02 and helium for this method A4.2.4/03. These two gases are inert. So this method is not an acceptable confirmatory technique.
Reliability	
Acceptability	Not acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.2.4	**Animal and human body fluids and tissues**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.2.4/04** Oullier J-P., Soun A. (1995) XXXX and metabolites (XXXX - Analytical determination method of plasma levels in micro-pigs XXXX no GLP 22 December 1995 (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	
2.2	**GLP**	No	
2.3	**Deviations**	N/A	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	None	
3.1.2	Cleanup	Liquid extraction using toluene	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC)	**X**
3.2.2	Detector	Electron capture	
3.2.3	Standard(s)	Internal	
3.2.4	Interfering substance(s)	Negligible matrix interference	
3.3	**Linearity**		
3.3.1	Calibration range	A series was checked at the following plasma concentrations of Fipronil, XXXX: 1, 2, 5 and 10 ng/mL (low calibration range) 1, 10, 25 and 100 ng/mL (high calibration range)	

Section A4.2.4		Animal and human body fluids and tissues	
3.3.2	Number of measurements	4-5	
3.3.3	Linearity	Correlation coefficient fipronil r^2=0.9997 XXXX r^2=0.9985 XXXX r^2=0.9929	x
3.4	**Specificity: interfering substances**	Negligible matrix interference	
3.5	**Recovery rates at different levels**	Plasma samples were spiked with Fipronil, XXXX at the following concentrations: 1, 5, 10 and 100 ng/mL for Fipronil, XXXX Each concentration was measured four or five times. fipronil 82 - 110 % XXXX 64 - 130 % XXXX 95 - 120 %	
3.5.1	Relative standard deviation	Not reported; calculated values were as follows: fipronil 2.3 - 5.5 % XXXX 2.5 - 20.3 % XXXX 1.9 - 5.0 %	
3.6	**Limit of determination**	1.0 ng/mL for each compound	x
3.7	**Precision**		
3.7.1	Repeatability	See 3.5.1	
3.7.2	Independent laboratory validation	Not required	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	The analytical method is used to determine residues of the active substance Fipronil and its metabolites (XXXX) in micro-pig plasma by means of GC-ECD after liquid extraction using toluene. Quantification is carried out by internal standardisation.	
4.2	**Conclusion**	The results obtained during the validation are within the limit of acceptance (± 20% for precision and 80-120% for accuracy) for Fipronil and XXXX.	x
4.2.1	Reliability		
4.2.2	Deficiencies	The study was not performed in compliance with GLP. Furthermore, the results obtained during the validation are not within the limit of acceptance (± 20% for precision and 80-120% for accuracy).	x

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2010
Materials and methods	**3.2.1 Separation method** The details on the chromatographic method are missing: The column is DB225, 15 m, 0.530 mm i.d., 1 µm film thickness - Carrier gas: helium , 9.5 mL/min - Temperature programme: 200°C, 2°C/min, 245°C hold 5 min. - Injection: 225°C **3.3.3 Linearity** There are only results for the low calibration range. For the low calibration range: fipronil r^2=0.9997 XXXX r^2=~~*0.9985*~~ 0.9929 XXXX r^2=~~*0.9929*~~ 0.9985 For the high calibration range: fipronil r^2=0.9996 XXXX r^2=0.9979 XXXX r^2=0.9999 **3.6 Limit of determination** This is not the limit of determination (LOQ) but the limit of detection (LOD) ~~*1.0 ng/mL for each compound*~~ Fipronil: LOQ=1ng/mL XXXX: LOQ=5ng/mL XXXX : LOQ=10ng/mL **4.2 Conclusion** According to TNG on data requirements, the limit of acceptance for accuracy is ~~*80-120%*~~ 70-110%
Conclusion	The validated method A4.2.4/02 uses a DB5 MS column (non polar), this method A4.2.4/04 uses a DB 225 column (polar).The polarity between these two columns are different. So this method is an acceptable confirmatory technique. Agree with applicant's version with above amendments.
Reliability	2 (not GLP)
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks

Section A4.3 **Annex Point IIA, IV.1**	**Analytical methods for residues in/on food or feedstuffs**

Section A4.3.1	**Analytical method for residues in plants**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A4.3.1/01** Fuchsbichler, G. (2001) Independent laboratory validation of method study XXXX for the determination of Fipronil and its metabolites XXXX in plants. XXXX 28 February 2001. (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	**X**
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Residues are extracted from plant matrices by macerating with a mixture of water/acetone	**X**
3.1.2	Cleanup	Gel permeation chromatography followed by silica gel chromatography	
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC)	**X**
3.2.2	Detector	Electron Capture Detector (ECD) and Mass Selective Detector (MSD)	**X**
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.02 to 0.002 mg/kg for each product	**X**
3.3.2	Number of measurements	5	**X**

Section A4.3.1	**Analytical method for residues in plants**	
3.3.3 Linearity	The linearity was found to be acceptable with r^2>0.991	
3.4 Specifity: interfering substances	None	
3.5 Recovery rates at different levels	For each type of compound at each fortification level the mean recovery was between 70 % and 110 % See Tables A4.3.1-1 to A4.3.1-5	
3.5.1 Relative standard deviation	For each type of compound at each fortification level RSD was lower that 20 % See Tables A4.3.1-1 to A4.3.1-5	
3.6 Limit of determination	Limit of quantification 0.002 mg/kg for each compound	
3.7 Precision		
3.7.1 Repeatability	See Tables A4.3.1-1 to A4.3.1-5	
3.7.2 Independent laboratory validation	Not applicable	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	Residues were extracted from the plant material with a water/acetone (1/2, v/v) mixture. The extract is partitioned into cyclohexane/ethyl acetate. For sunflower seeds, the residues were extracted with an acetonitrile/acetone (9/1; v/v) mixture. The extracts were worked up using Gel Permeation Chromatography (GPC) followed by silica gel column fractionation. For final determination Gas Chromatography (GC) was used. Quantification was performed by external standardisation using either an Electron Capture Detector (ECD) or a Mass Selective Detector (MSD).	
4.2 Conclusion	Specificity: Parent compound and its metabolites (XXXX) can be determined. The specificity of the method is provided by the use of a mass selective detector. Repeatability: See results of Relative Standard Deviation (RSD) – Tables A4.3.1-1 to A4.3.1-5. Limit of Quantification (LOQ): 0.002 mg/kg for each product Confirmatory method: not required since use of a mass selective detector Validation: The method was validated by preparing and analysing control samples as well as samples spiked at the limit of quantification (LOQ) and 10 x LOQ. Results obtained during the method validation are presented in the Tables A4.3.1-1 to A4.3.1-5 **Conclusion**: The multi-residue method DFG S19 was successfully independently validated for Fipronil and its metabolites (XXXX) in plant products.	**x** **x**
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.3.1-1 Maize

Maize			
	Fipronil	**XXXX**	**XXXX**
Control value	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)
0.002 mg/kg	102; 96; 91 105; 96 Mean* : 98% RSD: 5.0%	90; 87; 96 94; 84 Mean* : 90% RSD: 4.9%	92; 88; 88 98; 81 Mean* : 89% RSD: 6.2%
0.020 mg/kg	86; 86; 99 80; 73 Mean: 85% RSD: 10.1%	74; 74; 81 74; 66 Mean: 74% RSD: 6.4%	79; 79; 87 79; 87 Mean: 82% RSD: 4.8%

*Individual results obtained by GC/MSD analysis

Table A4.3.1-2 Potato

Potato			
	Fipronil	**XXXX**	**XXXX**
Control value	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)
0.002 mg/kg	80; 70; 96 103; 80; 90 Mean: 87% RSD: 13.8%	87; 78; 112 112; 81; 91 Mean: 94% RSD: 14.7%	93; 77; 103 113; 82; 88 Mean: 93% RSD: 3.2%
0.020 mg/kg	100; 88; 92 85; 103 Mean: 94% RSD: 7.4%	110; 114; 98 90; 106 Mean: 104% RSD: 8.3%	110; 105; 96 92; 105 Mean: 102% RSD: 6.5%

Table A4.3.1-3 Sunflower seed

Sunflower seed			
	Fipronil	**XXXX**	**XXXX**
Control value	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)
0.002 mg/kg	101; 104; 101 95; 100 Mean*: 100% RSD: 2.9%	107; 104; 105 107; 105 Mean*: 106% RSD: 1.1%	101; 97; 99 94; 97 Mean*: 98% RSD: 2.4%
0.020 mg/kg	66; 69; 75 70; 70 Mean: 70% RSD: 2.9	85; 91; 70 80; 70 Mean: 79% RSD: 10.5%	87; 101; 97 102; 102 Mean: 98% RSD: 5.8%

*Individual results obtained by GC/MSD analysis

Table A4.3.1-4 Bean

Bean			
	Fipronil	**XXXX**	**XXXX**
Control value	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)	< 30% LOQ (n = 2)
0.002 mg/kg	87; 90; 83 80; 90 Mean: 86% RSD: 4.6%	78; 82; 68 82; 82 Mean: 78% RSD: 6.9%	88; 92; 79 79; 88 Mean*: 85% RSD: 6.2%
0.020 mg/kg	95; 95; 99 91; 88 Mean: 94% RSD: 4.0%	99; 89; 108 99; 94 Mean: 98% RSD: 6.5%	81; 77; 86 77; 81 Mean: 80% RSD: 4.1%

Table A4.3.1-5 Additional validation results obtained via GC/MSD quantification

Substrate	Control value	Fipronil	XXXX	XXXX
Bean 0.002 mg/kg	< 30% LOQ (n = 1)	102; 104; 100 100; 96 Mean: 100% RSD: 2.6%	94; 91; 93 90; 97 Mean: 93% RSD: 2.6%	89; 99; 101 91; 92 Mean: 94% RSD: 5.0%
Potato 0.002 mg/kg	< 30% LOQ (n = 1)	105; 98; 103 99; 103; 97 Mean: 101% RSD: 2.9%	104; 100; 101 98; 96; 91 Mean: 98% RSD: 4.2%	89; 95; 97 89; 95; 94 Mean: 93% RSD: 3.3%
Sunflower seed 0.020 mg/kg	< 30% LOQ (n = 1)	95; 94; 93 92; 86 Mean: 92% RSD: 3.7%	93; 96; 94 95; 95 Mean: 95% RSD: 1.1%	95; 86; 82 86; 101 Mean: 90% RSD: 7.7%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	**2.1 Guideline study** Directive 96/46/EC and Guideline SANCO/825/00 rev.6 **3.1.1 Enrichment** *Residues* in beans, maize corn and potatoes *are extracted from plant matrices by macerating with a mixture of water/acetone* with subsequent partition into acetone/cyclohexane/ethyl acetate. Residues in sunflower seeds are extracted with acetonitrile/acetone. **3.2.1 Separation method** The details on the chromatographic method are missing: GC/ECD: - The column is DB-5 (30 m, 0.25 mm i.d., 0.25 µm film) - Carrier gaz: helium 4.6, 3,0 mL/min - Temperature programme: 100°C, 1 min then 15°C/min to 180°C, 1 min and 6°C/min to 250°C, 5 min - Injection: 1 µL, 240°C GC/MSD: - The column is DB-5 (30 m, 0.25 mm i.d., 0.25 µm film) - Carrier gaz: helium 5.3, 0.8 mL/min - Temperature programme: 100°C, 1 min then 10°C/min to 250°C, 10 min - Injection: 2 µL, 260°C **3.2.2 Detector** *Electron Capture Detector (ECD) and Mass Selective Detector (MSD)* ECD is not suitable at LOQ level in maize corn and sunflower beans (matrix effects disturb detector response). MSD is suitable for every matrix and level. **3.3.1 Calibration range** ~~*0.02 to 0.002 mg/kg*~~ 0.002 - 0.01 mg/*l for each product* **3.3.2 Number of measurements** ~~*5*~~ 6 **3.4 Recovery rates at different levels** Fortification levels: 0.02 to 0.002 mg/kg **4.2 Conclusion** *The specificity of the method is* ~~*provided by the use of*~~ confirmed by the analyses with *a mass selective detector.* ***Conclusion**: The multi-residue method DFG S19 was successfully independently validated for Fipronil and its metabolites (XXXX) in plant products.* The analyse by MSD is more precise than ECB, and necessary for low concentrations (0.02 mg/kg) in beans and potatoes.
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	The analyse by MSD is more precise than ECB, and necessary for low concentrations (0.02 mg/kg) in beans and potatoes.
	COMMENTS FROM …
Date	

Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Section A4.3.2	**Analytical method for food of animal origin**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**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 XXXX 21 June 1999. (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	**x**
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	~~Residues are extracted from animal matrices by macerating with a mixture of acetonitrile/acetone or water/acetone.~~ Residues in animal matrices were extracted as follows: - for fatty animal matrices (chicken egg, bovine fat), the extraction was performed with an acetonitrile/acetone (9/1, v/v) mixture - for non fatty animal matrices (bovine muscle, milk), the extraction was performed with a mixture of water/acetone (1/2, v/v). Addition of sodium chloride and ethyl acetate/cyclohexane liquid partition resulted in phase separation and partition of the pesticides in the upper organic phase (this step is used for bovine muscle and milk only).	**x**
3.1.2	Cleanup	~~Gel permeation chromatography followed by silica gel chromatography~~ The organic phase was concentrated and applied to Gel Permeation Chromatography (GPC). The eluate was then cleaned up through a silica gel column. The final sample extract was analysed by Gas Chromatography (GC) using an Electron Capture Detector (ECD) on a CP-Sil 8 CB MS capillary column.	**x**
3.2	**Detection**		
3.2.1	Separation method	Gas Chromatography (GC)	**x**

Section A4.3.2		**Analytical method for food of animal origin**	
3.2.2	Detector	Electron capture detector (ECD)	
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		**x**
3.3.1	Calibration range	0.02 to 0.002 mg/kg for each product	**x**
3.3.2	Number of measurements	5	**x**
3.3.3	Linearity	The linearity was found to be acceptable with $r^2>0.987$	**x**
3.4	**Specifity: interfering substances**	None	**x**
3.5	**Recovery rates at different levels**	For each type of compound at each fortification level the mean recovery was between 70 % and 110 % See Tables 4.3.2-1 to 4.3.2-5	**x**
3.5.1	Relative standard deviation	For each type of compound at each fortification level RSD was ≤ 20% See Tables 4.3.2-1 to 4.3.2-5	**x**
3.6	**Limit of determination**	0.002 mg/kg for each product (except in bovine fat)	
3.7	**Precision**		
3.7.1	Repeatability	See Tables 4.3.2-1 to 4.3.2-5	
3.7.2	Independent laboratory validation	Yes	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Residues in animal matrices were extracted as follows: - for fatty animal matrices (chicken egg, bovine fat), the extraction was performed with an acetonitrile/acetone (9/1, v/v) mixture - for non fatty animal matrices (bovine muscle, milk), the extraction was performed with a mixture of water/acetone (1/2, v/v). Addition of sodium chloride and ethyl acetate/cyclohexane liquid partition resulted in phase separation and partition of the pesticides in the upper organic phase (this step is used for bovine muscle and milk only). The organic phase was concentrated and applied to Gel Permeation Chromatography (GPC). The eluate was then cleaned up through a silica gel column. The final sample extract was analysed by Gas Chromatography (GC) using an Electron Capture Detector (ECD) on a CP-Sil 8 CB MS capillary column. The quantification was done by external standardisation.	

Section A4.3.2	**Analytical method for food of animal origin**	

4.2 Conclusion	Specificity: Parent compound and its metabolites (XXXX) can be determined due to the detection technique. Repeatability: see results of RSD (Relative Standard Deviation) below Limit of Quantification (LOQ): 0.002 mg/kg for each product (except in bovine fat) Confirmatory method: Yes, DB-210 capillary column. Validation: The method was validated by preparing and analysing control samples and samples spiked at the limit of quantification (LOQ) and 10 x LOQ. Results obtained during the method validation are presented in the Tables 4.3.2-1 to 4.3.2-5 **Conclusion**: The multi-residue method DFG S19 was successfully validated for Fipronil and its metabolites (XXXX) in bovine muscle, milk and egg	**x**
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.3.2-1 Bovine muscle

Bovine muscle				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)
0.002 mg/kg	105; 65; 75 100; 85 Mean:86% RSD: 20%	105; 105; 100 85; 80 Mean:95% RSD: 13%	100; 75; 95 95; 75 Mean:88% RSD: 14%	110; 95; 100 100; 70 Mean:95% RSD: 16%
0.020 mg/kg	95; 93; 103 70; 87 Mean: 90% RSD: 13%	94; 101; 106 93; 90 Mean: 97% RSD: 7%	95; 100; 107 77; 86 Mean: 93% RSD: 13%	106; 110; 117 110; 95 Mean: 108% RSD: 7%

Table A4.3.2-2 Bovine milk

Bovine milk				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)
0.002 mg/kg	105; 80; 85 100; 80 Mean:90% RSD: 13%	95; 75; 85 95; 80 Mean:86% RSD: 10%	105; 85; 95 105; 80 Mean:94% RSD: 12%	100; 100; 85 90; 70 Mean:89% RSD: 13%
0.020 mg/kg	87; 73; 84 85; 84 Mean: 83% RSD: 7%	82; 71; 82 83; 78 Mean: 79% RSD: 6%	84; 77; 80 83; 82 Mean: 81% RSD: 4%	95; 81; 86 86; 79 Mean: 85% RSD: 7%

Table A4.3.2-3 Bovine fat

Bovine fat				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)	<20% LOQ (n=2)
0.002 (mg/kg	90; 55; 65 Mean: 70% RSD: 26%	75; 75; 75 80; 60 Mean:73% RSD: 11%	70; 85; 65 85; 55 Mean:72% RSD: 18%	80; 100; 90 100; 60 Mean:86% RSD: 20%
0.020 mg/kg	88; 60; 79 76; 75 Mean: 76% RSD: 13%	107; 63; 84 66; 74 Mean: 79% RSD: 23%	99; 58; 89 75; 74 Mean: 79% RSD: 20%	89; 60; 77 74; 72 Mean: 74% RSD: 14%

Table A4.3.2-4 Chicken egg

Chicken egg				
	Fipronil	**XXXX**	**XXXX**	**XXXX**
Control value	<25% LOQ (n=2)	<25% LOQ (n=2)	<30% LOQ[b] (n=2)	<20% LOQ (n=2)
0.002 mg/kg	90; 80; 85 70; 90 Mean: 83% RSD: 10%	100; 100; 100 85; 90 Mean: 95% RSD: 7%	95; 80; 105 100; 80 Mean:92% RSD: 13%	90; 85; 85 95; 90 Mean: 89% RSD: 4%
0.020 mg/kg	54[a]; 93; 92 81; 76; 80 Mean: 84% RSD: 10%	41[a]; 76; 69 64; 60; 70 Mean: 84% RSD: 10%	49[a]; 98; 86 88; 90; 73 Mean: 87% RSD: 10%	50[a]; 94; 89 87; 79; 75 Mean: 85% RSD: 9%

Chicken egg[a]: the fortification at 0.020 mg/kg for XXXX could be excluded from the calculation of the average due to sample loss during sample preparation.

Chicken egg[b]: due to an interference at the retention time of the XXXX in control chicken eggs, the recovery rates for XXXX were corrected according to the control sample value.

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	
Materials and methods	**2.1 Guideline study** ~~*None available*~~ directive 96/46/CE and EU guidance document 8064/VI/97 rev4 **3.1.1 Enrichment** ~~*Residues are extracted from animal matrices by macerating with a mixture of acetonitrile/acetone or water/acetone.*~~ Residues in animal matrices were extracted as follows: - for chicken egg and bovine fat, the extraction was performed with an acetonitrile/acetone (9/1, v/v) mixture - for bovine muscleand milk, the extraction was performed with a mixture of water/acetone (1/2, v/v), followed by addition of sodium chloride and ethyl acetate/cyclohexane and liquid partition. **3.1.2 Cleanup** ~~*Gel permeation chromatography followed by silica gel chromatography*~~ The organic phase was concentrated and applied to Gel Permeation Chromatography (GPC). The eluate was then cleaned up through a silica gel column. The final sample extract was analysed by Gas Chromatography (GC) using an Electron Capture Detector (ECD) on a CP-Sil 8 CB MS capillary column. **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is CP-Sil 8 CB-MS (30 m, 0.25 mm i.d., 0.25 µm film) - Carrier gaz: helium, 15 psi - Temperature programme: 90°C for 2 min, then 30°C/min to 235°C, 2°C/min to 255°C and 30°C/min to 300°C, hold time 2 min - Injection: 2 µL, (120°C, 0.10 min, 180°C/min to 270°C, 2 min) **3.3 Linearity** The data for linearity are not presented in doc IV (quadratic calibration) **3.4 Specifity: interfering substances** None, except for XXXX in chicken egg. The signal must be corrected by subtracting the blank value (about 2xLOQ). The corrected recovery is good. **3.5 Recovery rates at different levels** *For each type of compound at each fortification level the mean recovery was between 70 % and 110 %* except for Fipronil in bovine fat at 0.002 mg/kg (70% after exclusion of 2 outliers). **3.5.1 Relative standard deviation** *For each type of compound at each fortification level RSD was ≤ 20%* except for XXXX in bovine fat at 0.02mg/kg (23%). **4.2 Conclusion** *The multi-residue method DFG S19 was successfully validated for Fipronil and its metabolites (XXXX) in bovine muscle, milk and* chicken *egg.* The method is

	not suitable to quantify fipronil and metabolits in bovine fat.
Conclusion	Adopt applicant's version with above amendments
Reliability	2 (linearity not given)
Acceptability	Acceptable except for bovine fat.
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.3.2	**Analytical method for food of animal origin**	

		1. REFERENCE	**Official use only**
1.1	**Reference**	**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 XXXX 11 January 2007. (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	**x**
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Residues are extracted from animal matrices: Milk and eggs, liver, muscle, kidney: using a mixture of methanol and water Fat: fat matrix is dissolved in isohexane and extracted using acetonitrile	
3.1.2	Cleanup	Milk and eggs: liquid/liquid partition against dichloromethane Liver, muscle and kidney: liquid/liquid partition against dichloromethane followed by a silicagel clean-up	
3.2	**Detection**		
3.2.1	Separation method	HPLC	**x**
3.2.2	Detector	MS/MS, monitoring per analyte two parent-daughter ion transitions	**x**
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	0.0005 to 0.005 mg/kg for each product	**x**
3.3.2	Number of measurements	10	

Section A4.3.2	**Analytical method for food of animal origin**	
3.3.3 Linearity	The linearity was found to be acceptable with r^2>0.9990	
3.4 Specifity: interfering substances	None	
3.5 Recovery rates at different levels	For each type of compound at each fortification level the mean recovery was between 70 % and 110 % (with the exception of one finding for fipronil for transition 2: 435→ 250 for liver: 68.8%) See Table A4.3.2-5	x
3.5.1 Relative standard deviation	For each type of compound at each fortification level RSD was ≤ 20%	
3.6 Limit of determination	0.0005 mg/kg for each product	
3.7 Precision		
3.7.1 Repeatability	See Table A4.3.2-5	
3.7.2 Independent laboratory validation	Yes, see 4.3.2/03	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods	<u>Extraction and clean-up</u> For milk and eggs: BAS 350 I and its metabolites are extracted from milk and eggs using a mixture of methanol and water. For clean-up a liquid/liquid partition against dichloromethane was used. For liver, muscle, kidney: BAS 350 I and its metabolites are extracted from liver, muscle, kidney using a mixture of methanol and water. For clean-up a liquid/liquid partition against dichloromethane followed by a silicagel clean-up. For fat: For the determination of BAS 350 I and its metabolites the fat matrix is dissolved in isohexane and extracted using acetonitrile. <u>Final determination</u> The final determination of BAS 350 I and its metabolites XXXX is performed by HPLC-MS/MS (all matrices) monitoring per analyte two parent-daughter ion transitions. The LOQ is 0.0005 mg/kg.	
4.2 Conclusion	The results of the validation data show that BASF method No. 568/0 is suitable to determine Fipronil and its metabolites XXXX in animal matrices. The limit of quantification was defined by the lowest fortification level successfully tested, which was 0.0005 mg/kg in animal matrices. The repetition of the HPLC-MS/MS measurement after storage of the final volume (5-7 days) leads to equal results. This proves that the analytes are stable over the time investigated if kept under refrigerated conditions.	
4.2.1 Reliability	1	
4.2.2 Deficiencies	None	

Table A4.3.2-5

Matrix	n	1. transition					2. transition				
		max	min	mean (%)	SD (±)	CV (%)	max	min	Mean (%)	SD (±)	CV (%)
Fipronil		**435 → 330 (quantifier)**					**435 → 250**				
liver	10	89.6	71.6	78.5	5.5	7.0	79.9	62.5	68.8	4.3	6.3
fat	10	100.5	90.6	95.9	3.2	3.3	109.4	89.6	98.6	6.3	6.4
muscle	10	88.6	70.4	78.9	4.9	6.2	88.8	70.3	79.4	5.9	7.4
milk	10	84.1	73.6	78.5	3.9	5.0	91.6	74.9	80.8	5.6	7.0
kidney	10	95.5	81.6	88.3	4.6	5.3	97.7	76.3	86.3	6.8	7.9
egg	10	82.8	73.7	78.9	3.3	4.2	95.6	62.9	84.1	9.4	11.2
XXXX		**451 → 415 (quantifier)**					**451 → 282**				
liver	10	93.0	78.9	85.4	4.1	4.8	91.2	75.4	83.9	4.6	5.5
fat	10	106.0	93.8	98.2	3.7	3.8	102.4	95.3	99.2	2.4	2.4
muscle	10	109.2	93.1	101.1	5.4	5.4	109.4	92.5	101.9	5.9	5.8
milk	10	103.4	90.0	95.9	5.1	5.4	103.2	87.7	95.3	4.6	4.8
kidney	10	104.9	91.3	97.3	4.7	4.8	99.3	86.7	93.8	4.2	4.5
egg	10	86.1	73.8	78.3	4.0	5.1	87.3	71.3	78.9	5.5	7.0
XXXX		**419 → 383 (quantifier)**					**419 → 262**				
liver	10	88.3	75.1	82.6	3.7	4.5	90.0	71.9	83.1	5.0	6.1
fat	10	108.4	92.6	100.6	4.9	4.9	103.0	89.9	97.0	4.8	5.0
muscle	10	92.4	83.6	88.4	3.2	3.6	99.0	82.4	91.7	5.1	5.6
milk	10	88.0	80.5	84.5	3.0	3.5	98.5	81.1	85.1	5.1	5.9
kidney	10	98.1	84.3	91.7	5.0	5.4	99.3	86.6	90.9	3.3	3.6
egg	10	84.8	70.7	76.0	4.9	6.4	87.8	68.9	80.4	6.1	7.6
XXXX		**387 → 351 (quantifier)**					**387 → 282**				
liver	10	90.2	73.1	80.6	4.6	5.7	96.5	68.3	80.8	9.0	11.2
fat	10	101.2	90.6	95.7	3.8	4.0	115.4	81.8	101.6	11.8	11.6
muscle	10	96.3	84.0	89.2	5.1	5.7	97.7	77.3	87.0	7.0	8.1
milk	10	96.9	83.2	87.8	4.8	5.4	99.8	70.4	83.8	9.7	11.5
kidney	10	120.5	84.9	94.6	10.3	10.9	130.3	84.8	99.1	13.2	13.3
egg	10	88.6	77.3	83.5	3.8	4.6	99.0	68.4	80.2	10.3	12.8
XXXX		**453 → 304 (quantifier)**					**453 → 272**				
liver	10	102.4	72.4	85.5	11.6	13.6	101.6	69.5	81.5	10.1	12.4
fat	10	110.3	88.6	100.3	7.2	7.2	106.4	73.4	91.4	10.2	11.2
muscle	10	89.7	68.5	79.5	6.3	7.9	95.8	68.8	82.2	9.6	11.7
milk	10	101.8	72.7	87.5	8.9	10.2	112.4	68.8	88.2	15.8	17.9
kidney	10	108.8	76.9	91.1	11.9	13.1	105.8	77.6	88.0	8.3	9.4
egg	10	94.5	81.8	87.9	4.4	5.0	111.3	78.4	92.6	10.7	11.5

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	**2.1 Guideline study** ~~*None*~~ OPPTS 860.1340, SANCO 825/00 rev.6, Directive 96/46/CE, SANCO/3029/99 rev.4 **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is Betasil C18, 100×2 mm, 5 µm particle size - The mobile phase A is Millipore water/Formic acid and the mobile phase B is Methanol/Formic acid - The injection is 10 µL (or higher) and the flow rate is 600 µL/min **3.2.2 Detector** Add the type of ionization: negative ion mode **3.3.1 Calibration range** ~~*0.0005 to 0.005 mg/kg*~~ 0.002 to 0.1ng/ml *for each product* **3.4 Recovery rates at different levels** Fortification levels : 0.0005 and 0.005 mg/kg The values in table A4.3.2-5 correspond to data obtained with the two fortification levels (0.0005 and 0.005 mg/kg, 5 values for each). It would be better to differentiate values from each fortification levels. Nevertheless no significant differences were observed in the report.
Conclusion	Adopt with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	Table A4.3.2-5 should differentiate data from each fortification levels. Nevertheless, it does not compromise the validity and reliability of the method.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A4.3.2	**Analytical method for food of animal origin**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**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 XXXX 02 December 2005 (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None available	**X**
2.2	**GLP**	Yes	
2.3	**Deviations**	Not relevant	
		3. MATERIALS AND METHODS	
3.1	**Preliminary treatment**		
3.1.1	Enrichment	Fat matrix is dissolved in isohexane and extracted using acetonitrile	
3.1.2	Cleanup		
3.2	**Detection**		
3.2.1	Separation method	HPLC	**X**
3.2.2	Detector	MS/MS, monitoring per analyte two parent-daughter ion transitions	**X**
3.2.3	Standard(s)	External	
3.2.4	Interfering substance(s)	None	
3.3	**Linearity**		
3.3.1	Calibration range	1.5 to 0.010 ng/ml for each product	
3.3.2	Number of measurements	5	
3.3.3	Linearity	Correlation coefficients were always better than r>0.998	
3.4	**Specifity: interfering substances**	None	
3.5	**Recovery rates at different levels**	The independent laboratory validation acceptance criteria (recovery rates 70 % and 110 %) were met.	**X**

Section A4.3.2	**Analytical method for food of animal origin**	

3.5.1	Relative standard deviation	For each type of compound at each fortification level RSD was ≤ 20%	
3.6	**Limit of determination**	0.0005 mg/kg for each product	
3.7	**Precision**		
3.7.1	Repeatability	See Table A4.3.2-6	
3.7.2	Independent laboratory validation	Not applicable	
		4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Extraction and clean-up For the determination of BAS 350 I and its metabolite XXXX, the fat matrix is dissolved in isohexane and extracted using acetonitrile. Final determination The final determination of BAS 350 I and its metabolites XXXX is performed by HPLC-MS/MS, monitoring per analyte two parent-daughter ion transitions. The LOQ is 0.0005 mg/kg. Validation The independent laboratory validation (ILV) was completed successfully by analysing 2 blank control specimens, 5 replicate specimens fortified at LOQ or 0.0005 mg/kg per analyte, 5 replicate specimens fortified at 10xLOQ (0.005 mg/kg), and 5 replicate specimens fortified at 20xLOQ (0.01mg/kg). The validation acceptance criteria were met with average recovery rates ranging from 86% to 109%, relative standard deviations ≤ 11%, and negligible interference signals in control specimens.	
4.2	**Conclusion**	PTRL Europe performed the independent laboratory validation (ILV) of fipronil (BAS 350 I) and its metabolite XXXX in animal matrices, exemplified with bovine fat. The method was shown to be highly selective, as it includes two-parent daughter ion transitions per analyte, and it yields accurate and repeatable results. No contacts to the developers of the original method were necessary. It is concluded that BASF method 568/0 fulfils the reproducibility requirements as defined in the EU Directive 91/414/EEC and is, therefore, applicable as enforcement method.	
4.2.1	Reliability	1	
4.2.2	Deficiencies	None	

Table A4.3.2-6

Results	**Fortification Level**	**Fipronil**		**XXXX**	
		435 m/z → 330 m/z	**435 m/z → 415 m/z**	**451 m/z → 415 m/z**	**451 m/z → 282 m/z**
Average	LOQ	104%	102%	109%	107%
RSD	0.0005 mg/kg	11%	10%	4%	2%
Average	10xLOQ	103%	103%	103%	103%
RSD	0.005 mg/kg	2%	2%	3%	2%
Average	20xLOQ	87%	86%	93%	92%
RSD	0.01 mg/kg	4%	4%	5%	5%
Overall average		98%	97%	102%	101%
Overall RSD		11%	10%	8%	7%

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	**2.1 Guideline study** ~~*None*~~ SANCO/825/00 rev.7 **3.2.1 Separation method** The details on the chromatographic method are missing: - The column is Luna RP-C18, 50 mm× 2.0 mm, 5 µm particle size - The mobile phase A is 0.1% formic acid in water and the mobile phase B is 0.1% formic acid in methanol - The injection is 50 µL and the flow rate is 200 µL/min **3.2.2 Detector** Add the type of ionization: TurboIonSpray - negative ion mode **3.5 Recovery rates at different levels** Fortification levels : 0.0005, 0.005 and 0.01 mg/kg
Conclusion	Adopt applicant's version with above amendments
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A5 **Annex Point IIA, V**		**Effectiveness Against Target Organisms and Intended Uses**	
Subsection **(Annex Point)**			**Official use only**
5.1	**Function** **(IIA5.1)**	Insecticide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected** **(IIA5.2)**		
5.2.1	Organism(s) to be controlled (IIA5.2)	German *(Blattella germanica)*, Oriental *(Blatta orientalis)* American *(Periplaneta americana)* Cockroaches both as nymphs and adults.	
5.2.2	Products, organisms or objects to be protected (IIA5.2)	For the protection of humans from the nuisance of cockroach infestations and the diseases which they may carry.	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used** **(IIA5.3)**		
5.3.1	Effects on target organisms (IIA5.3)	Insect death following ingestion or contact. See section 5.4.1 Mode of Action.	
5.3.2	Likely concentrations at which the A.S. will be used (IIA5.3)	0.05 % w/w	
	PT18	Ready to use crawling insect gel products to contain 0.05%w/w active substance	
5.4	**Mode of action (including time delay)** **(IIA5.4)**		
5.4.1	Mode of action	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.	

Section A5 **Annex Point IIA, V**		**Effectiveness Against Target Organisms and Intended Uses**	
5.4.2	Time delay	Fipronil when used as Goliath Gel is shown to have an overnight effect. Allowing insects eating bait (e.g. cockroach) to consume sufficient to allow a lethal dose to be ingested. For insects controlled by contact activity (e.g. termites) the delayed activity allows for contaminated individuals to return to the nest and transfer compound to ensure the entire colony is destroyed	**x**
5.5	**Field of use envisaged (IIA5.5)**		
	MG01: Disinfectants, general biocidal products	Not applicable	
	MG02: Preservatives	Not applicable	
	MG03: Pest control	PT 18 – Insecticide for the control of cockroaches and other crawling insects	**x**
	MG04: Other biocidal products	Not applicable	
	Further specification	Not applicable	
5.6	**User (IIA5.6)**		
	Industrial	None	
	Professional	Cockroach Gel: Professional Pest Control Operators to apply through a specialist gel gun into cracks and crevices in domestic, industrial and public premises	
	General public	None	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**		

Section A5 **Annex Point IIA, V**	**Effectiveness Against Target Organisms and Intended Uses**	
5.7.1 Development of resistance	(Anonymous, 2006): As with most insecticides, overuse of fipronil can lead to the development of resistance in target insects. The first instances of true, field level loss of activity were seen in Southeast Asia as early as 1996. In these areas, fipronil resistance developed in diamondback moth (*Plutella xylostella*) within three years of the introduction of the product. The high intrinsic activity of fipronil and lack of effective compounds in the marketplace led growers in countries such as Thailand to use fipronil up to 40 times per year on cruciferous crops. Field resistance to fipronil has now also been detected in rice leaf beetle in Japan (Ueno 2005) and two species of rice stem borer in China (Cao 2004 and Jiang 2004). To date, BASF has received no reports of field failures against cockroaches due to resistance to fipronil. Outside Europe, dieldrin was used extensively for cockroach control for many years and there are reports in the literature of fipronil susceptibility in cockroaches being correlated with a history of dieldrin use (Holbrook 2003). Because cyclodienes and fipronil share a common binding site within the pore of the GABA receptor, binding-site-based cyclodiene resistance might be presumed to confer a high level of cross-resistance to fipronil, but this is in fact not the case. A mutation in the binding site can affect compounds acting at that site quite differently, and although this particular resistance mechanism confers very high levels of "resistance to dieldrin" and is therefore known as Rdl, the level of resistance to most other compounds acting at that site, including some cyclodienes and fipronil, is much lower (Kristensen 2005, Cole 1993, Cole 1995, Scott 1997). Additionally, fipronil acts potently at two types of insect-specific glutamate-gated chloride channel (Ikeda 2003, Zhao 2004), which may help to further mitigate the effects of dieldrin-resistant GABA receptors. Fipronil has been shown to be only very weakly cross-resistant with chlorpyrifos and deltamethrin (Salmeron 2003), but in the same paper fipronil is recommended as a tool for resistance management for cockroaches. Fipronil was also shown to not be cross-resistant with pyrethroids in a multi-resistant strain of cockroaches (Wei 2001) and again, was recommended as a tool in an integrated resistance management program. Overall, fipronil appears to not be cross-resistant with other classes of chemistry.	**x**

Section A5 **Annex Point IIA, V**	**Effectiveness Against Target Organisms and Intended Uses**	
5.7.2 Management strategies	There are many cockroach control products available commercially which it is anticipated that PCO's will use and that cockroaches will be exposed to several active ingredients. Fipronil resistance appears to have a fitness cost and low heritability. By skipping a generation (or even better, two) between fipronil applications, the insects that are susceptible are allowed to resurge, with the weaker, resistant insects receding into the background. By following a relatively high rate strategy, the fewest number of insects with the resistant gene survive. The gene for fipronil resistance appears to be recessive, so in order to be expressed two resistant insects must mate. By allowing fewer survivors the chance of this happening is decreased. Combined with a rotational strategy mentioned above, the chances that homozygous insects will survive until the next fipronil application is made are significantly reduced. Resistance is an inevitable consequence of the overuse of any insecticide. However, a judicious management plan can slow and even reverse the development of resistance in a population. (Anonymous, 2006): Studies on the heritability of fipronil resistance have shown that the resistance is at least incompletely recessive and controlled by a single locus (Sayyed 2004, 2005). The combination of this with the lowered reproductive fitness offers a good opportunity to use a rotational insecticide regime as the cornerstone of a resistance management plan for fipronil. As an internal resistance monitoring program showed in Thailand, when selection pressure was removed from the local populations of *P. xylostella*, the LC_{50} values returned to almost the same levels as when the program had started, indicating the return of susceptibility in the populations. The final recommendations for an integrated resistance management plan for fipronil should contain the following elements: • **Rotation of mode of action** - use other modes of action to remove resistant individuals from a population • **Proper dosage** - Fipronil should be applied at the correct dosage so as to achieve as complete a kill as possible. This "high dosage" strategy means that fewer insects will survive to pass on the resistance genes. This is especially effective when combined with a rotational scheme. • **Proper timing** - Target the most susceptible stage of the pest and target the timing of applications for best control. • **Monitoring** - Information from resistance monitoring programs allows early detection of problems and gives information for correct decision making There are currently cockroach control products, both sprays and gels, based on numerous different chemistries, including avermectin, imidacloprid, chlorpyrifos and numerous pyrethroids. The diversity of modes of action from this array of products should allow for an excellent rotational program to minimize the development of resistance, both to fipronil and other compounds as well.	

Section A5 **Annex Point IIA, V**	**Effectiveness Against Target Organisms and Intended Uses**	
5.8 Likely tonnage to be placed on the market per year (IIA5.8)	Confidential data. Please see Annex 1.	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22/01/08
Comments	**Sections 5.4.2 and 5.5** The efficacy data provided in the dossier supports the label claim for cockroach control but not for other pest organisms (e.g. no efficacy studies were provided on termites in DOC IIIA & DOC IIIB). Then, it is rather confusing to mention some information on *termites* in the dossier. In addition, the term" *crawling insects*" which refers to a general label must be replaced by the term "*cockroaches*" which is more appropriate. **Section 5.7.1.** Some statements regarding cross-resistance issue between fipronil and cyclodienes are not properly captured from the literature (i.e. *"binding-site-based cyclodiene resistance might be presumed to confer a high level of cross-resistance to fipronil, but this is in fact not the case*). Indeed, many publications have reported varying level of cross resistance between fipronil and cyclodienes (dieldrin) among different insect groups including cockroaches (Kristensen et al., 2005), flies (Cole et al., 1995, Hossie et al., 1995, Scott & Wen, 1997), fleas (Bass et al.,2004) and mosquitoes (Brooke et al. 2000; Kolaczinski et al., 2001; Davari et al., 2007). As dieldrin resistance is expect to be present in field populations, one cannot exclude that a long term use of fipronil may select existing resistance gene (ie Rdl mutation and detoxifying enzymes) and then lead to operational failure. Consequently, some statements have been revised in DOC IIB to better capture the scientific data on cross resistance. One should note however, that the applicant proposed appropriate resistance management strategies in the dossier.
Conclusion	Despite an underestimation of the issue relating to cross-resistance of fipronil with other insecticides, the efficacy of the active substance is demonstrated and supported the label claims for cockroach control in field situations.
Acceptability	Study report ; Accepted
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A5.9/01** Kaakeh W, Reid B L, and Bennett G W (1997) The toxicity of fipronil to German and American cockroaches 229 -237) (XXXX)	
1.2	**Data protection**		
1.2.1	Data owner	Published	
1.2.2	Companies with letter of access	Not applicable	
1.2.3	Criteria for data protection	Not applicable	
1.3	**Guideline study**	No	
1.4	**Deviations**	Not applicable	
		2. METHOD	
2.1	**Test material**	Fipronil technical 0.5% fipronil bait (no further details given)	
2.1.1	Lot/Batch number	Not given	
2.1.2	Specification	Not given	
2.1.2.1	Description	Not given	
2.1.2.2	Purity	98.6%	
2.1.2.3	Stability	Stable	
2.2	**Reference substance**	Chlorpyrifos 99% - topical and oral (Petri dish) application. Combat Roach Killing System (1.65% hydromethylon) - oral (population chamber) and bait attractivity tests. Raid Roach Bait (0.528% chlorpyrifos) - oral (population chamber) application.	
2.2.1	Method of analysis for reference substance	Not applicable	
2.3	**Testing procedure**		
2.3.1	Test population / inoculum / test organism	German cockroach *Blattella germanica* (Johnson Wax strain). American cockroach *Periplaneta americana* (field caught strain). Adult males (topical toxicity test). Adult males and 4th instar nymphs (oral toxicity – Petri dish bioassay). Adult males and females and large nymphs of *P. americana* only (oral toxicity – population chamber bioassay). Adult males and females for the bait attractancy test.	**X**

2.3.2 Test system	Topical Toxicity 100 mm plastic Petri dishes containing a water vial. Oral Toxicity – Petri dish bioassay Ventilated plastic boxes (19x13x10cm) containing a cardboard harbourage, water vial and test material. Oral Toxicity – Population chamber bioassay Escape proof chamber with a floor area of 1 m^2 containing a harbourage, water and an alternative food source (laboratory diet). Bait Attractancy test 90 cm arenas containing a harbourage, water and alternative food sources (laboratory diet, jelly).
2.3.3 Application of TS	Topical Toxicity 1 µl of insecticide solutions applied topically to the intercoxal spaces on the ventral mesothorax of anaesthetised insects. For *B. germanica* the solutions contained 1, 2, 3, 4, 10, 20, 30 or 40 ppm fipronil or chlorpyrifos in acetone. For *P. gmericana* the solutions contained 40, 100, 400, 600, 800, 1000, or 4000 ppm fipronil or chlorpyrifos in acetone. Oral Toxicity – Petri dish bioassay Baits were prepared by finely grinding laboratory diet and pelleting using insecticide in acetone solutions. For *B. germanica* the baits contained 1000, 500, 250 or 100 ppm fipronil or chlorpyrifos For *P. americana* the baits contained 10,000, 5,000, 2,500 or 1000 ppm fipronil or chlorpyrifos Oral Toxicity – Population chamber bioassay After the insects had been acclimatised in the chamber for 24 hours the test baits were introduced in a bait station made from a Petri dish. Bait Attractancy test After the insects had been acclimatised in the chamber for 24 hours the test baits were introduced into the arenas.

2.3.4	Test conditions	Topical Toxicity Ambient room conditions Oral Toxicity – Petri dish bioassay Ambient room conditions Oral Toxicity – Population chamber bioassay Temperature: 27°C Relative humidity: 50% Photoperiod: 12 hours light/12 hours dark Bait Attractancy test Ambient room conditions	
2.3.5	Duration of the test / Exposure time	Topical Toxicity 72 hours Oral Toxicity – Petri dish bioassay In one test the insects were exposed to the treated bait for 24 hours after which it was removed and replaced with untreated bait for the remainder of the test period (72 hours). In a further test the insects were exposed to the treated bait for 72 hours. Oral Toxicity – Population chamber bioassay 14 days Bait Attractancy test 3 hours	
2.3.6	Number of replicates performed	Topical Toxicity 3 per concentration (10 *B germanica* and 4 *P americana* per replicate) Oral Toxicity – Petri dish bioassay Three for each concentration/exposure combination. Oral Toxicity – Population chamber bioassay Three each for the test material and control baits Bait Attractancy test Not recorded	**x** **x**
2.3.7	Controls	Topical Toxicity Acetone only Oral Toxicity – Petri dish bioassay Untreated pelleted bait Oral Toxicity – Population chamber bioassay None Bait Attractancy test None	

2.4 Examination

2.4.1	Effect investigated	Mortality in the toxicity tests and no of insects feeding at a particular food stuff in the attractancy test
2.4.2	Method for recording / scoring of the effect	Visual observation
2.4.3	Intervals of examination	<u>Topical Toxicity</u> 6, 24, 48 and 72 hours after treatment <u>Oral Toxicity – Petri dish bioassay</u> 8, 16, 24, 48 and 72 hours after introduction of the bait <u>Oral Toxicity – Population chamber bioassay</u> daily <u>Bait Attractancy test</u> 10 minute intervals
2.4.4	Statistics	<u>Topical Toxicity</u> LD_{50} – probit analysis <u>Oral Toxicity – Petri dish bioassay</u> SAS ANOVA <u>Oral Toxicity – Population chamber bioassay</u> LD_{50} – probit analysis Daily mortality SAS ANOVA <u>Bait Attractancy test</u> Data were converted to an attractancy ratio by dividing the number of cockroaches at the test material by the number of insects feeding on the laboratory diet.
2.4.5	Post monitoring of the test organism	None

3. RESULTS

3.1	**Efficacy**	<u>Topical Toxicity</u> See Table A5.9.1-1 <u>Oral Toxicity – Petri dish bioassay</u> See Table A5.9.1-2 <u>Oral Toxicity – Population chamber bioassay</u> See Table A5.9.1-3

		Bait Attractancy test *P americana* were more attracted to fipronil than to combat or other alternative foods. The attractancy ratios (bait laboratory diet) for fipronil and combat baits were 2:1 and 1.5:1 respectively. *B. germanica* were attracted similarly to fipronil and Combat baits. The attractancy ratios were 1.3:1 and 1:1 for fipronil and Combat respectively.	x
3.2	**Efficacy limiting factors**		
3.2.1	Occurrences of resistances	None seen in the tests reported in this study. However the *B. germanica* strain used (Johnson Wax strain) had been isolated from a wild population before the widespread use of synthetic organic insecticides and is susceptible. Any cross-resistance with, for instance, dieldrin would not therefore be apparent. The *P. americana* used in the tests were wild caught at the time of the study but no information on susceptibility is reported.	x
3.2.2	Other limiting factors	The oral Toxicity – Petri dish bioassay shows that nymphs are less susceptible to fipronil (and chlorpyrifos) than adult American cockroaches.	
		4. RELEVANCE OF THE RESULTS COMPARED TO FIELD CONDITIONS	
4.1	**Reasons for laboratory testing**	Initial tests to determine the intrinsic toxicity of fipronil to cockroaches in comparison with commercially available insecticides	
4.2	**Intended actual scale of biocide application**	Domestic premises (including kitchens) Food handling areas (i.e. for food processing, storage and preparation) such as food manufacturing premises, commercial kitchens, restaurants, food stores, warehouses, retail outlets etc. Public Buildings such as hotels, hospitals, prisons, theatres Commercial and industrial premises such as factories, shops, workshops, aircraft, vehicles, railway stock and ships.	x
4.3	**Relevance compared to field conditions**		
4.3.1	Application method	The bait was presented as a pellet rather than drops of gel as is the current commercial recommendation. However the test was designed investigate the topical and oral toxicity of fipronil to cockroaches	
4.3.2	Test organism	Species as are found naturally occurring	
4.3.3	Observed effect	Overnight effect and mortality as required in real use	
4.4	**Relevance for read-across**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Topical Toxicity The toxicity of fipronil and chlorpyrifos was determined by topical application to Adult male cockroaches (Blattella germanica and	

Periplaneta americana).

One µl of insecticide solutions containing 1, 2, 3, 4, 10 20 30 or 40 ppm of fipronil or chlorpyrifos were applied to the inter-coxal spaces of the ventral mesothorax of *B. germanica* briefly anaesthetised with CO_2 to facilitate handling. *P. americana* were similarly treated with solutions of 40, 100, 400, 600, 800, 1000 and 4000 ppm of fipronil and chlorpyrifos. After treatment the cockroaches were placed in groups in Petri dishes.

Control cockroaches were treated with acetone alone. Each concentration and control was replicated 3 times and mortality was observed at 6, 24 48 and 72 hours following treatment.

Oral Toxicity – Petri dish bioassay

Diets containing varying concentrations of either fipronil or chlorpyrifos (100, 250, 500 or 1000 ppm for *B. germanica* and 1000, 2500, 5000 or 10,000 ppm for *P. americana*) were prepared by finely grinding laboratory diet and reconstituting to pellets with an acetone solution of insecticide and water. Control pellets were made using an acetone blank and water.

Ten adult males or 4th instar nymphs of *B. germanica* (4 for P. americana) were placed in a plastic box (19 x 13 x 10cm) covered with a ventilated lid. A water vial, tent cardboard harbourage and a sing pellet of the test diet (2 pellets for *P. americana* were paced in each box. The insects had previously been starved for 24 hours before testing.

Two exposures were conducted in the study. In one group the insects were allowed continuous exposure to the treated pellets. In the other, insects were given the treated died for 24 hours after which it was replaced with non-treated pellets.

Observations were made at 8, 16, 24, 48, and 72 hours. Each concentration-by-exposure combination was replicated three times.

Oral Toxicity – Population chamber bioassay

The efficacy of an experimental bait containing 0.05% fipronil and the commercially available baits Combat Roach Killing System (1.65% hydromethylnon) and Raid Roach Bait (0.528% chlorpyrifos) against P. *americana* were compared. The tests were conducted in a climatically controlled environment in test arenas of area 1m^2. The arenas contained a harbourage food (Wayne rodent block) and water and the insects (35 large nymphs and 15 mixed adults) were acclimatised in the arenas for 24 hours before treatment.

Four tablets of each bait were glued to the inverted lid of a Petri dish. The bottom of the Petri dish, covered from inside with foil, was placed on the top of the inverted lid to make a bait stations. After the acclimatisation period these were placed in the arenas.

Each treatment was replicated three times. Daily observations for mortality were made for 14 days.

Bait Attractancy test
Evaluation of the attractancy to *B. germanica* and *P americana* was conducted in a 90 cm diameter confinement with varying foodstuffs (water, untreated laboratory diet and jelly) and the two candidate baits (fipronil and Combat – containing hydramethylon) distributed in a 75cm circular array. Adult cockroaches (25 individuals per sex) were released and offered harbourage, laboratory diet and water in the centre of the arena at least 24 hours prior to the test's beginning.

All tests were conducted at night under infrared illumination.. Beginning one hour into the scotophase the number of foraging cockroaches at each food or bait matrix location was recorded at 10 minute intervals for 120 minutes. Three replicates were conducted for each species. Data were converted to an attractancy ratio for each candidate bait matrix by dividing the total number of cockroaches at each bait matrix by the total number of cockroaches at the laboratory diet.

5.2 Reliability 1

5.3 Assessment of efficacy, data analysis and interpretation

Topical Toxicity

LD_{50}'s were estimated by probit analysis; indication of significant differences was determined by non overlap of the 95% fiducial limit. Dose was expressed as weight of insecticide (µg)/average weight of an adult male (g). Incidences of control mortality we automatically corrected by Abbott's transformation.

At 72 hours fipronil was more toxic than chlorpyrifos both species of species of cockroach (0.03µg/g and 0.06µg/g respectively for *B. germanica* and 0.02µg/g and 0.16µg/g respectively for *P. americana*).

Oral Toxicity – Petri dish bioassay

Both fipronil and chlorpyrifos were toxic to adult male cockroaches of both species at all concentrations tested. Fipronil caused significantly higher nymphal mortality than chlorpyrifos 48 hours after exposure in both feeding bioassays.

Oral Toxicity – Population chamber bioassay

Fipronil was more effective and faster in killing P. americana than Raid Roach and Combat baits. The speed of kill may be ranked as follows fipronil<Raid<Combat.

One day after baiting fipronil induced mortality was significantly higher than those caused by Raid and Combat. At the end of the 14 day test period, the percentage mortality increased sharply and reached 96.5, 93.4 and 84.6 for fipronil, Raid and Combat baits respectively.

Bait Attractancy test

P americana were more attracted to fipronil than to combat or other alternative foods. The attractancy ratios (bait: laboratory diet) for fipronil and combat baits were 2:1 and 1.5:1 respectively.

B. germanica were attracted similarly to fipronil and Combat baits. The attractancy ratios were 1.3:1 and 1:1 for fipronil and Combat respectively.

5.4 Conclusion

5.5 Proposed efficacy specification

Goliath Gel is effective against German and American cockroaches.

Table A5.9.1-1 Topical toxicity of fipronil and chlorpyrifos

Cockroach species	Insecticide	Time (hours)	LD50 (µg/g)	Confidence Limits
B germanica	Fipronil	6	0.36	-0 – 0
		24	0.12	0.01 – 0.17
		48	0.04	0.01 – 0.07
		72	0.03	0.02 – 0.04
	Chlorpyrifos	6	7.74	2.10 - >100
		24	1.60	0.74 – 9.37
		48	0.28	0.07 - >100
		72	0.06	0.02 – 0.11
P americana	Fipronil	6	0.15	0.10 – 0.21
		24	0.08	0.05 – 0.12
		48	0.03	0.02 – 0.05
		72	0.02	-0 - 0
	Chlorpyrifos	6	0.33	0.28 – 0.37
		24	0.32	0.27 – 0.36
		48	0.29	0.25 – 0.32
		72	0.16	0.02 – 0.35

Table A5.9.1-2 Results from the oral (Petri-dish) study

Species	Diet concentration	Feeding bioassay	Insecticide	% mortality of males					% mortality of 4th instars				
				8h	16h	24h	48h	72h	8h	16h	24h	48h	72h
B germanica	100	Abb	Fipronil	7	17	100	100	100	0	20*	77	80	87
			Chlorpyrifos	0	3	67	90	90	0	0	77	80	80
		Cont	Fipronil	0	33**	100**	100	100	3	17	57	87	100
			Chlorpyrifos	0	0	57	97	100	0	7	37	80	100
	250	Abb	Fipronil	0	100	100	100	100	0	20**	67	77	80
			Chlorpyrifos	0	7	30	83	87	0	0	47	47	47
		Cont	Fipronil	0	100**	100	100	100	0	17**	57	77	93
			Chlorpyrifos	0	0	53	90	100	0	0	40	70	87
	500	Abb	Fipronil	13	100**	100	100	100	0	20	70	87	93
			Chlorpyrifos	0	3	70	90	100	0	7	67	73	73
		Cont	Fipronil	0	100	100	100	100	3	17	63	90**	100**
			Chlorpyrifos	0	3	77	97	100	0	3	30	57	87
	1000	Abb	Fipronil	27*	100**	100	100	100	0	37*	70*	90**	93**
			Chlorpyrifos	0	3	77	93	97	0	33	50	57	57
		Cont	Fipronil	13.3	100**	100	100	100	3	23	87	90	90
			Chlorpyrifos	0	0	100	100	100	0	10	53	67	87
P americana	1000	Abb	Fipronil	0	0	8	42	42	0	0	17	58*	58**
			Chlorpyrifos	0	0	8	25	33	0	0	0	17	25
		Cont	Fipronil	0	0	17	50	50	0	8	25	83	92*
			Chlorpyrifos	0	0	8	42	42	0	0	8	33	42
	2500	Abb	Fipronil	0	0	17	42	42	0	0	25	50	58
			Chlorpyrifos	0	0	17	33	42	0	0	8	17	33
		Cont	Fipronil	0	0	17	50	67	0	8	33	92**	100**
			Chlorpyrifos	0	0	8	42	60	0	0	8	33	42
	5000	Abb	Fipronil	0	8	50	58	75	0	17	50**	83*	83*
			Chlorpyrifos	0	0	33	80	58	0	0	8	25	33
		Cont	Fipronil	0	25	58	75	83	0	33	75**	100**	100**
			Chlorpyrifos	0	17	50	75	75	0	0	17	42	50

Species	Diet concentration	Feeding bioassay	Insecticide	% mortality of males					% mortality of 4th instars				
				8h	16h	24h	48h	72h	8h	16h	24h	48h	72h
	10,000	Abb	Fipronil	0	0	42	67	75	0	8	50**	75*	92
			Chlorpyrifos	0	0	33	42	50	0	8	17	33	42
		Cont	Fipronil	0	13	63	88	88	0	17	58	100**	100**
			Chlorpyrifos	0	8	42	67	75	0	0	25	42	58

Abb – abbreviated test (insects exposed for 24 hours); cont – continuous test (insects exposed for 72 hours);
* and ** indicates significant differences at the 1 and 5% level for each species, insecticide concentration and feeding bioassay.

Table A.5.9.13 Results from the oral (Population chamber) study

Bait	LT_{50} days	% mortality at day			
		1	3	7	14
Fipronil 0.05%	0.8	46.0	88.0	95.3	96.5
Chlorpyrifos 0.528%	2.4	28.0	55.4	78.6	93.4
Hydramethylon (1.65%	7.6	3.3	9.8	39.3	84.6

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22/01/08
Objectives	**The objectives of the study were;** - to determine the topical toxicity of fipronil against adults *B. germanica* and *P. americana;* - to determine the oral toxicity of fipronil against males and 4th instars of both cockroach species; - to evaluate untreated, fipronil and Combat bait matrices for their attractancy to both cockroach species.
methods	**2-3. Testing procedure (2-3-1)** It was misleading to mention that the *P. americana* strain used for bioassays was caught in the field whereas it was taken from the laboratory (number of generations not reported). **2-3-2. Topical toxicity.** Age of *P. americana* used for topical toxicity was not mentioned unlike *B. germanica* (1-2 weeks). **2-3-2. Tests for oral toxicity in population chamber.** Bioassay were done with formulated products (0.05% fipronil, Combat Roach Killing System, 1.65% hydramethylnon and Raid Roach bait 0.528% chlorpyrifos) and not with the active substance. The same trend was noted with the attractancy test carried out on *B. germanica* and *P. americana* with two candidate baits. Consequently, these data should have been reported in the DOC IIIB (effect of biocidal product).instead of DOC IIIA **2-3-6 Number of replicates.** The number of tested cockroaches was rather low , i.e. 4 specimens of *P. americana* per replicate for both topical and oral toxicity, which is not sufficient to ensure appropriate comparison. 3-1: "Combat bait" is short term for the reference substance "Combat Roach Killing System used in the study.
results	**3-2-1 Occurrences of resistance.** The applicant did not fully capture the author's interpretation regarding the cross resistance pattern between dieldrin and fipronil. Indeed, the published document clearly states that cross resistance between dieldrin and fipronil in Drophila may occur in *B. germanica*.
General Comments	In general, this study was carried out following appropriate methodology and statistics.
Conclusion	This study demonstrated the efficacy of fipronil against two major species of cockroaches to be controlled. Moreover, this study showed equal or better performances of fipronil compared to chlorpyrifos, a reference product for cockroach control (WHO, 2006.1). In spite of the few number individuals used in some experiments, this study can be accepted as a proof of evidence that fipronil is effective against these two targeted species.
Reliability	2
Acceptability	*Study report* : Accepted

Remarks	Section 4.2. The product may be applied in occupied premises, except during cooking activities. It is recommended to apply GOLIATH GEL in cracks and crevices, or in concealed locations inaccessible to man or domestic animals: behind refrigerators cupboards and shelves, under kitchen appliances (the application on hoods has not been evaluated), in electrical control boxes, voids and ducting and under bathroom fixtures etc. Spots should not be applied in areas where it will become submersed or likely to be removed by routine cleaning.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1	**Acute toxicity**
Annex Point IIA, VI.6.1	

Section A6.1.1	**Acute oral toxicity**
Annex Point IIA,VI.6.1.1	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.1/01** XXXX. (1988a) Acute oral toxicity to rats of M&B 46,030. XXXX 17 October 1988. (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 to Annex 1.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EEC 79/831/EEC (1979) OECD 401 (1987)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil (M&B 46,030)	
3.1.1	Lot/Batch number	IGB 444	
3.1.2	Specification	Deviating from specification given in section 2 as follows : the purity is lower than the minimum of 95% in the current specification.	
3.1.2.1	Description	Green solid	
3.1.2.2	Purity	93%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Crl: CD(SD)	X
3.2.3	Source	XXXX	
3.2.4	Sex	Male/female	
3.2.5	Age/weight at study initiation	Age: 5 – 7 weeks weight: Males: 110 – 138 g Females:100 – 126 g	X
3.2.6	Number of animals per group	10 (5 male and 5 female)	
3.2.7	Control animals	No	X
3.3	**Administration/ Exposure**	Oral	
3.3.1	Post exposure period	14 days	
3.3.2	Type	Gavage	
3.3.3	Concentration	Gavage at 50, 80, 126 & 200 mg/kg bw	

Section A6.1.1 **Annex Point IIA,VI.6.1.1**	**Acute oral toxicity**	
3.3.4 Vehicle	Moistened with corn oil	
3.3.5 Concentration in vehicle	0.50, 0.80, 1.26 & 2.00% w/v	
3.3.6 Total volume applied	10 ml/kg bodyweight	
3.3.7 Controls	None	x
3.4 Examinations	Clinical observations, necropsy	x
3.5 Method of determination of LD_{50}	Finney	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Clinical signs	Principle clinical signs included : Pilo-erection, diarrhoea, hunched posture and abnormal gait (waddling) for the majority of animals in all groups with lethargy for the majority of animals treated at 80 mg/kg bw and above decreased respiration rate, pallor of the extremities and/or ptosis for a single male dosed at 80 mg/kg bw and all rats treated with 200 mg/kg bw clonic convulsions and prostration preceding death, for two males and one female respectively dosed at 200 mg/kg bw. The signs were generally observed within 5 hours of dosing and recovery was seen by Day 6 in all animals surviving to termination.	
4.2 Pathology	Terminal autopsy findings were commonly found to be normal. However hydronephrosis was apparent in one male and one female rat dosed at 80 mg/kg bw. As a low incidence of this abnormality occurs spontaneously amongst untreated rats of this strain at this laboratory, the finding was not considered to be related to treatment.	
4.3 Other	Low bodyweight gains were recorded on day 8 for up to two females at each dose level and for all males surviving treatment. With the exception of a single female dosed at 50 mg/kg bw which showed a slightly low bodyweight gain, all rats achieved anticipated bodyweight gains during the second week of the study.	
4.4 LD_{50}	**Males: 92 mg/kg bw** Females: 103 mg/kg bw Combined sexes: 97 mg/kg bw	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fipronil was administered by gavage to rats according to the following design in order to assess the acute oral toxicity. Groups of 5 male and 5 female Sprague Dawley rats were given a single oral dose by gavage of Fipronil diluted in corn oil (w/v) and administered at a dose volume of 10 ml/kg. Dose levels were 50, 80, 126, and 200 mg/kg bw. They were selected on the basis of results in a preliminary study which indicated that the acute median lethal dose was about 100 mg/kg bw. Animals were observed frequently on the day of dosing for mortality and clinical signs of toxicity and twice daily thereafter for 14 days. Individual body weights were recorded just prior to dosing, weekly thereafter and at termination. All animals were examined macroscopically post mortem.	

Section A6.1.1 **Annex Point IIA,VI.6.1.1**	**Acute oral toxicity**	
	The rats were about 5 to 7 weeks of age and weighed 110 to 138 g (males) and 100 to 126 g (females) on the day of dosing. They were acclimatised for 1 week prior to dosing.	X
5.2 Results and discussion	Mortality: (see Table A6.1.1-1) Mortality was seen at dose levels of 80 mg/kg bw and above in males and females. Deaths occurred within four hours of dosing until Day 3 of the observation period. males only : 92 (64 – 128) mg/kg bw females only : 103 (73 – 141) mg/kg bw males + females : 97 (76 – 122) mg/kg bw. Clinical signs (see Table A6.1.1-2) The principle clinical signs included: pilo-erection, diarrhoea, hunched posture and abnormal gait (waddling) for the majority of animals in all groups with lethargy for the majority of animals treated at 80 mg/kg bw and above; decreased respiration rate, pallor of the extremities and/or ptosis for a single male dosed at 80 mg/kg bw and all rats treated with 200 mg/kg bw; clonic convulsions and prostration preceding death, for two males and one female respectively dosed at 200 mg/kg bw. The signs were generally observed within 5 hours of dosing and recovery was seen by Day 6 in all animals surviving to termination. Bodyweight Low bodyweight gains were recorded on Day 8 for up to two females at each dose level and for all males surviving treatment. All surviving rats achieved anticipated bodyweight gains during the second week of the study apart from one female dosed at 50 mg/kg bw which showed a slightly low gain. Post mortem examination There were no gross necropsy findings that were considered to be treatment-related.	
5.3 Conclusion	The acute oral LD_{50} to rats with 95% confidence limits was calculated to be: males only : 92 (64 – 128) mg/kg bw females only : 103 (73 – 141) mg/kg bw males + females : 97 (76 – 122) mg/kg bw.	
5.3.1 Reliability	2 (The purity of the material used in the study was lower than the current specification)	
5.3.2 Deficiencies	Yes: The material used in this study had a purity of 93%. Since the study was conducted the specification for technical fipronil has been modified such that the minimum purity is 95%. This slight difference in purity is unlikely to have a significant impact on the results of the study and would not justify the use of further animals to undertake a further study. The non GLP study conducted in the mouse using material with a purity of 95.3% resulted in LD_{50} of 95 mg/kg bodyweight (Males 98 and females 91) supporting the findings of the rat study.	

Table A6.1.1-1 Acute oral toxicity in rats – mortality

Dose [mg/kg bw]	Number of dead/number of investigated			Time of death (range)	Observations
	Males	Females	Combined sexes		
50	0 / 5	0 / 5	0 / 10	na	See below
80	2 / 5	2 / 5	4 / 10	~~5-24 hours~~ 2 days	
126	4 / 5	4 / 5	8 / 10	~~5 hours to 3 days~~ 2 days to 3 days	
200	5 / 5	4 / 5	9 / 10	~~5 hours to 2 days~~ 4 hours to 2 days	
LD_{50}	**92 mg/kg bw**	**103 mg/kg bw**	**97 mg/kg bw**		

X
X
X

Table A6.1.1-2 Acute oral toxicity in rats – clinical signs

Sign	Dose level (mg/kg bw)							
	50		80		126		200	
	M	F	M	F	M	F	M	F
Piloerection	5	5	5	5	5	5	5	5
Diarrhoea	5	5	5	5	5	5	5	5
Hunched posture	5	5	5	5	5	5	3	5
Waddling gait	5	5	5	5	5	5	3	5
Lethargy	0	0	5	5	5	5	3	5
Decreased respiratory rate	0	0	1	0	0	0	5	5
Ptosis	0	0	0	0	0	0	5	5
Pallor of extremities	0	0	1	0	0	0	5	5
Clonic convulsions	0	0	0	0	0	0	2	0
Prostrate	0	0	0	0	0	0	0	1

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with the applicant's version **Revisions/amendments:** 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor. 3.2.2 Strain: ~~*Crl: CD(SD)*~~ Crl: CD(SD)BR 3.2.5 Age/weight at study initiation and 5.1 Materials and methods: ~~*Age: 5 – 7 weeks*~~ Age: 4 – 6 weeks 3.2.7 Control animals - 3.3.7 Controls: ~~*No*~~ historic controls 3.4 Examinations: ~~*Clinical observations, necropsy*~~ Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days the animals were observed one in the morning and again at the end of the experimental day. Clinical signs were recorded at each observation. Approximate time of death of individual rats was recorded. The nature, severity, approximate time of onset and duration of each toxic sign were recorded. Body weight was recorded on Days 1, 8 and 15. All animals on the study were killed on Day 15 by carbon dioxide asphyxiation and were subjected to a macroscopic post mortem examination, which consisted of opening the cranial, abdominal and thoracic cavities. The macroscopic appearance of abnormal organs when present was recorded.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rats**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.2/01** XXXX. (1988b) Acute dermal toxicity to rats of M&B 46,030. XXXX 11 October 1988 (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes 79/831/EEC (1979) OECD 402 (1987)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil (M&B 46,030)	
3.1.1	Lot/Batch number	IGB 444	
3.1.2	Specification	Deviating from specification given in section 2 as follows : the purity is lower than the minimum of 95% in the current specification.	
3.1.2.1	Description	Green solid	
3.1.2.2	Purity	93%	
3.1.2.3	Stability	Stable	**X**
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD (Crl : CD(SD) BR	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	7 – 10 weeks of age weight: Males: 232 – 250 g Females: 201 – 214 g	
3.2.6	Number of animals per group	10 (5 males and 5 females)	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	**X**
3.3.1	Post exposure period	14 days	
3.3.2	Area covered	10% of body surface	
3.3.3	Occlusion	Occlusive (impervious) dressing	
3.3.4	Vehicle	Distilled water	
3.3.5	Concentration in vehicle	90.9%	

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rats**

3.3.6	Total volume applied	2.2 ml/kg bw	
3.3.7	Duration of exposure	24 h	
3.3.8	Removal of test substance	Warm water	
3.3.9	Controls	None	
3.4	**Examinations**	Animals were observed soon after dosing and at frequent intervals for the remainder of Day 1. On subsequent days the animals were observed one in the morning and again at the end of the experimental day. Clinical signs were recorded at each observation. Body weight was recorded on Days 1, 8 and 15. All animals on the study were killed on Day 15 by cervical dislocation and were subjected to a macroscopic post mortem examination, which consisted of opening the abdominal and thoracic cavities. The macroscopic appearance of abnormal organs when present was recorded.	**x**
3.5	**Method of determination of LD_{50}**	Limit test	
3.6	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	There were no signs of systemic reaction to treatment. Sites of application of the test substance showed no irritation reactions or other dermal changes. Slightly low bodyweight gains were recorded on Day 8 for a single male rat and on Day 15 for a single female. One female showed a minor bodyweight loss during the first week of the observation period. Other rats achieved anticipated bodyweight gains throughout the study.	
4.2	**Pathology**	Terminal autopsy findings were normal	
4.3	**Other**		
4.4	**LD_{50}**	There were no deaths following a single derma dose of 2.0 g/kg bw, i.e. the acute dermal LD_{50} in rats is greater than 2000 mg/kg bw.	

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rats**

		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil was administered topically to rats according to the following design in order to assess the acute dermal toxicity. A group of 5 male and 5 female Sprague Dawley rats were given a single topical application of 2000 mg/kg bw Fipronil prepared as a 90.9% w/v concentration in distilled water and administered at a volume of 2.2 ml/kg bw. It was applied to clipped, intact dorsal skin for 24 hours under an occlusive (impervious) dressing. At the end of the exposure period, the dressing was removed and the skin washed with warm water to remove any remaining test substance then blotted dry. Animals were observed frequently on the day of dosing for mortality and clinical signs of toxicity and twice daily thereafter for 14 days. Individual body weights were recorded just prior to dosing, weekly thereafter and at death. All animals were examined macroscopically post mortem. The rats were about 9 to 12 weeks of age and weighed 220 to 250 g (males) and 201 to 214 g (females) on the day of dosing. They were acclimatised for approximately 2 weeks prior to dosing.	x
5.2	**Results and discussion**	There were no deaths and no treatment-related systemic clinical signs. No irritation or other dermal responses were seen at the application sites. Overall, bodyweight gain was unaffected by treatment although slightly low gains were recorded for one male on Day 8 and for one female on Day 15. One female showed minor bodyweight loss during the first week post-treatment. There were no treatment-related gross necropsy findings.	
5.3	**Conclusion**	The acute dermal LD_{50} of Fipronil in rats is greater than 2000 mg/kg bw	
5.3.1	Reliability	2 (The purity of the material used in the study was lower than the current specification)	
5.3.2	Deficiencies	Yes: The material used in this study had a purity of 93%. Since the study was conducted the specification for technical fipronil has been modified such that the minimum purity is 95%. This slight difference in purity is unlikely to have a significant impact on the results of the study and would not justify the use of further animals to undertake a further study.	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with the applicant's version **Revisions/amendments:** 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor. 3.3 Administration/exposure: dose: 2000 mg/kg bw 3.4 Examination: The nature, severity, approximate time of onset and duration of each toxic sign were recorded.; the treated areas of skin were examined daily for signs of dermal irritation.
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version **Revisions/amendments:** 5.1 Materials and methods: ~~*9 to 12 weeks of age*~~ 7 to 10 weeks of age and ~~*220 to 250 g (males)*~~ 232 to 250g
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rabbits**

					Official use only
		1. REFERENCE			
1.1	**Reference**	**A6.1.2/02** XXXX. (1992) M&B 46,030: Acute percutaneous toxicity study in the rabbit. 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 to Annex 1			
		2. GUIDELINES AND QUALITY ASSURANCE			
2.1	**Guideline study**	Yes USEPS (=EPA) FIFRA 81-2 (1984)			
2.2	**GLP**	Yes			
2.3	**Deviations**	No			
		3. MATERIALS AND METHODS			
3.1	**Test material**	As given in Section 2			**X**
3.1.1	Lot/Batch number	78/GC/90			
3.1.2	Specification	As given in section 2			**X**
3.1.2.1	Description	White powder			
3.1.2.2	Purity	96.7%			
3.1.2.3	Stability	Stable			
3.2	**Test Animals**				
3.2.1	Species	Rabbit			
3.2.2	Strain	New Zealand White			
3.2.3	Source	XXXX			
3.2.4	Sex	Male and female			
3.2.5	Age/weight at study initiation	12 – 18 weeks of age weight:	Males:	2.1 – 3.0 kg Females: 2.3 – 2.9 kg	
3.2.6	Number of animals per group	10 (5 males and 5 females)			
3.2.7	Control animals	No			
3.3	**Administration/ Exposure**	Dermal			**X**
3.3.1	Post exposure period	14 days (extended to 28 days at the two low dose levels because of delayed signs of toxicity and delayed deaths at the higher dose levels).			
3.3.2	Area covered	As large an area as possible of the whole trunk.			
3.3.3	Occlusion	Occlusive (impervious) dressing			
3.3.4	Vehicle	Corn oil			
3.3.5	Concentration in vehicle	Doses were administered on the basis of weight of test material/kg of animal weight. Weights of and corn oil for each individual animal are recorded in the report.			**X**
3.3.6	Total volume applied	As above			**X**

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rabbits**	
3.3.7 Duration of exposure	24h	
3.3.8 Removal of test substance	Not removed	x
3.3.9 Controls	None	
3.4 Examinations	Signs of toxicity, mortality and necropsy	x
3.5 Method of determination of LD_{50}	Thompson, W. R. (1947): Use of moving averages and interpolation to estimate medium-effective dose. Weil, C. S. (1983): Economical LD_{50} and slope determination.	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Clinical signs	(See Table A6.1.2.2-2) Several affected surviviors recovered within 2 to 14 days one female recovered after 28 days.	
4.2 Pathology	Necropsy of the rabbits that died revealed liquid in the thoracic cavities, discoloured lungs (pink to dark red), lungs with patches (tan, red and/or brown), lungs with a rough surface-texture and a moderate to large amount of blood in the urine (positive by HEMASTIX® reagent Strips).	
4.3 Other	Mortality (See Table A6.1.2.2-1)	
4.4 LD_{50}	The acute dermal LD_{50} of Fipronil to the rabbit was Male 445 mg/kg bw Females 354 mg/kg bw Combined sexes 354 mg/kg bw	x
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fipronil was administered topically to rabbit skin according to the following design in order to assess its acute dermal toxicity. Groups of 5 male and 5 female New Zealand White rabbits were given a single topical application of 100, 250, 500, 1000 or 2000 mg/kg bw Fipronil, moistened with corn oil, to the intact, clipped dorsal skin of each animal for 24 hours under an occlusive (impervious) dressing. The dressing was removed at the end of the treatment period and skin responses assessed over 14 days or 28 days post application. The observation period was extended to 28 days at the two low dose levels because of delayed signs of toxicity and delayed deaths at the higher dose levels. Rabbits were observed frequently on the day of administration and at least once thereafter for clinical signs and mortality. Individual bodyweights were recorded on the day of dosing and on Days 7 and 14 after dosing or at death. They were also recorded at 21 and 28 days where applicable. The rabbits were approximately 12 to 18 weeks of age and weighed 2.1 to 3.0 kg (males) and 2.3 to 2.9 kg (females) on the day of treatment. They were acclimatised for at least 5 days before dosing.	x
5.2 Results and discussion	Mortality (see Table A6.1.2-1) There was only one survivor at each of the two highest dose levels, 1000 and 2000 mg/kg bw and only three males survived treatment with 500 mg/kg bw. At 250 mg/kg bw, one male and two females died but there were no deaths at 100 mg/kg bw. All deaths occurred between Days 5 and 14 of the observation period.	x

Section A6.1.2 **Annex Point IIA, VI.6.1.2**	**Acute dermal toxicity** **– in rabbits**	
	Clinical signs and dermal reactions (see Table A6.1.2-2) The principal clinical signs of toxicity were clonic convulsions, spasms, tremors, perioral and perinasal red staining, diarrhoea and emaciation. The clonic convulsions, seen in both decedents and surviving animals at all except the lowest dose level (100 mg/kg bw), were delayed. Although of relatively short duration, they were initially observed on Days 3 to 9 post dosing but occurred several times throughout the day. Several affected survivors recovered within 2 to 14 days although one female recovered only at Day 28. There were no dermal reactions. Body weight and necroscopsy All rabbits except one female given 100 mg/kg lost bodyweight during the first week of the observation period. Losses were marked at 250 mg/kg and above. However from the second week onwards surviving rabbits gained weight. Necropsy of the rabbits that died revealed liquid in the thoracic cavities, discoloured lungs (pink to dark red), lungs with patches (tan, red and/or brown), lungs with a rough surface-texture and a moderate to large amount of blood in the urine. Necropsy of the survivors revealed dark red lungs (in 2 animals), dark red areas of the lungs (in 1 animal), bright red lungs with grey areas (in 1 rabbit), dark purple kidneys, an excessive amount of blood in the kidneys (in 1 rabbit), enlarged kidneys with pitted surface (in 2 rabbits) and enlarged spleen (1 animal).	**x**
5.3 Conclusion	**The acute dermal LD_{50} of Fipronil to the rabbit was:** Males: 445 mg/kg bw Females: 354 mg/kg bw Combined sexes: 354 mg/kg bw	**x**
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	**x**

Table ~~A6.1.2-1~~ A6.1.2.2-1 Acute dermal toxicity – mortality X

Dose [mg/kg bw]	**Number of dead/number of investigated**			**Time of death (range) days**	**Observations**
	M	**F**	**M&F**		
100	0/5	0/5	0/10	n.a	See table below
250	2/5	1/5	3/10	10 – ~~11~~ 12	
500	2/5	5/5	7/10	7 – 14	
1000	5/5	4/5	9/10	5 – 14	
2000	4/5	5/5	9/10	5 – 12	
LD_{50} value	**445 mg/kg bw**	**354 mg/kg bw**	**354 mg/kg bw**		

X

X

Table ~~A6.1.2-2~~ A6.1.2.2-2 Acute Dermal Toxicity – clinical signs

Sign	Dose level (mg/kg bw)									
	100		250		500		1000		2000	
	M	F	M	F	M	F	M	F	M	F
Emaciation	0	0	2	2	~~2~~ 3	4	~~2~~ 3	4	3	3
Clonic convulsions	0	0	2	2	2	5	4	1	2	1
Spasms	0	0	1	2	0	0	2	0	1	0
Tremors	0	0	0	0	0	0	~~1~~ 2	0	1	1
Perioral and perinasal red staining	0	0	0	1	1	2	2	2	0	3
Vocalisation	0	0	0	1	0	0	~~1~~ 2	0	0	0
Prostration	0	0	0	0	1	0	0	0	0	0
Diarrhoea	0	~~0~~ 2	0	0	1	1	0	~~0~~ 1	0	1
Sluggishness	0	0	1	0	0	0	0	0	0	0
Audible breathing	0	0	1	0	0	0	0	0	0	0
Hyperactivity	1	0	0	0	0	0	0	0	0	0
Salivation	0	0	0	0	0	0	0	0	0	1

X

X

X

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with the applicant's version **Revisions/amendments:** 3.1 Test material: ~~*as given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*as given in section 2*~~ none 3.3 Administration/exposure: dose: 100, 250, 500, 1000 and 2000 mg/kg bw 3.4 Examinations: *Signs of toxicity, mortality and necropsy* Treated rabbits were observed frequently for signs of toxic effect on the first day and twice a day thereafter. The time of death was also recorded. Weights were recorded on the day of dosing and at 7 and 14 days after dosing or at death. All rabbits were necropsied after death or sacrifice. 3.3.5 Concentration in vehicle: ~~*Doses were administered on the basis of weight of test material/kg of animal weight. Weights of and corn oil for each individual animal are recorded in the report*~~. no information 3.3.6 Total volume applied: ~~*as above*~~ no information 3.3.8 Removal of test substance: ~~*not removed*~~ All coverings were removed after a 24-hour contact period with water or other appropriate (relatively non-toxic) solvent..
Results and discussion	Agree with the applicant's version **Revisions/amendments:** 4.4 LD_{50}; 5.3 Conclusion: Combined sexes: *354 mg/kg bw* It represents the lowest value of the measured LD_{50}; corresponding of the females' LD_{50}.
Conclusion	Agree with the applicant's version **Revisions/amendments:** 5.1 Materials and methods: ~~*at least once thereafter*~~ twice a day thereafter (except on weekends or holidays when they were examined for death alone). 5.2 Results and discussion: *Mortality* ~~*(see Table A6.1.2-1)*~~ (see Table A6.1.2.2-1) ~~*. At 250 mg/kg bw, one male and two females died*~~ . At 250 mg/kg bw, two males and one female died *Clinical signs and dermal reactions* ~~*(see Table A6.1.2-2)*~~ (see Table A6.1.2.2-2) 5.3 Conclusion: *The acute dermal LD_{50} of Fipronil to the rabbit* with 95% confidence limits was: *Males:* *445* (200 to 980) *mg/kg bw* *Females:* *354* (200 to 620) *mg/kg bw* *Combined sexes:* ~~*354*~~ (210 to 600) *mg/kg bw* 5.3.2 Deficiencies: ~~*None*~~ no information about the purity and the total volume applied
Reliability	2

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

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with Fipronil technical**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.3/01** XXXX (1991) M&B 46030: Acute inhalation toxicity study in the rat. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 81-3 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**x**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**x**
3.1.2.1	Description	A slightly yellow powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	**x**
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD Strain (remote Sprague-Dawley origin)	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Males and females 7 – 12 weeks old Males 243 – 283g Females 213 – 247 g	
3.2.6	Number of animals per group	10 – 5 males and 5 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Post exposure period	14 days	
3.3.2 – 3.3.7		Not applicable (sections specific to acute oral toxicity studies)	
3.3.8	Concentrations	Nominal concentrations 0.456, 0.766 & 2.134 mg/l Analytical concentrations 0.259, 0.523 & 0.929 mg/l	
3.3.9	Particle size	The mean proportion of particles of inhalable size (< 6 µm Equivalent Aerodynamic Diameter) derived from cascade impactor samples were Group 1 29.7% (SD 8.2) Group 2 45.0% (SD 2.0) Group 3 40.0% (SD 5.9)	**x**

3.3.10 Type or preparation of particles	Dust aerosol (unmilled)	
3.3.11 Type of exposure	nose only	
3.3.12 Vehicle	None	
3.3.13 Concentration in vehicle	n.a	
3.3.14 Duration of exposure	4 h	
3.3.15 Controls	Vehicle only	x
3.4 Examinations	**Signs:** The animals were observed immediately before exposure, 15 and 30 minutes after the start of exposure and at 30-min intervals for the remainder of the exposure period. Following return to their cages, the animals were observed at 30-min intervals during the first three hours after exposure. Subsequently they were examined twice daily until completion of 14 days of observation. The type and time of onset and duration of reactions to treatment were recorded. **Mortality:** Animals which were severely debilitated were isolated to prevent cannibalism and observed carefully at least twice daily so that they could be found as soon as possible after death minimising the degree of autolysis. Bodyweight: Each rat was weighed daily from the day of delivery until the end of the observation period. **Euthanasia:** Rats that survived the observation period were placed under deep sodium pentobarbitone anaesthesia by intraperitoneal injection and killed by rapid exsanguinations. All rats killed on completion of the observation period were subjected to necropsy with a minimum of delay. Particular attention was paid to the frontal and nasal areas, the lungs and pleural surfaces. The lungs, liver and kidneys were weighed and the larynx, lungs, liver and kidneys were reserved.	x
3.5 Method of determination of LD_{50}	Finney	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Clinical signs	<u>Mortality</u> All deaths occurred during the 48 hours immediately following exposure. At 0.259 mg/l, one female was found dead on Day 2 of the observation period. At 0.523 mg/l, one male and one female were found dead during the first 24 hours immediately following exposure and one male and one female was found dead on Day 2. At 0.929 mg/l, three males were found dead during the first 24 hours following exposure, and one male and three females were found dead on Day 2 of the observation period.	

		Clinical signs During exposure, increased or decreased respiratory rate, struggling in the restraint tube and wet fur were observed. All observations were considered to be non-specific responses to exposure to a particulate atmosphere. During the three hours immediately following the exposure, hypothermia was recorded for all animals of 0.523 mg/l and some of the animals 0.259 mg/l. Other signs recorded during this period were hunched posture, tremors and vocalisation when handled. In addition, brown staining of the fur and wet fur were observed for most animals. Signs that persisted or that developed during Days 2 to 14 of the observation period included hunched posture, piloerection, penis mutilation, yellow staining, salivation, tremors, convulsions ataxia, hairloss and vocalisation when handled.	
4.2	**Pathology**	The external appearance at necropsy was consistent with the clinical observation of discolouration of the fur. There were no internal findings that were attributed to treatment. Organ weight analysis indicated that the lung weights of decedents were higher than expected. There were no other organ weight changes.	
4.3	**Other**	Bodyweight All surviving animals lost, or gained a minimal amount of bodyweight during the first 24 hours after exposure. Surviving animals exposed to 0.929 mg/l lost weight for a further three days following exposure. Survivors exposed to 0.259 mg/l and 0.523 mg/l lost bodyweight or gained no weight for a further two days. Subsequently, the observed weight gain was within the expected range.	
4.4	**LD_{50}**	0.682 mg/l of air for male and female rats combined	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil (unmilled) was administered as a dust aerosol to the rat as a 4-hour continuous snout-only exposure in order to assess the acute inhalation toxicity. Three groups of 5 male and 5 female Sprague Dawley rats were exposed by the nose only continuously for 4 hours to analytically determined atmosphere concentrations of 0.259, 0.523 or 0.929 mg/litre of the test substance. Atmosphere measurements included the achieved gravimetric concentration, particle size distribution and chamber temperature and humidity. Animals were observed for mortality and clinical signs immediately before, 15 and 30 minutes after the start of exposure and at 30-minute intervals for the rest of the 4-h exposure period. For three hours immediately after the end of the exposure period they were observed at 30-minute intervals. Subsequently, twice daily observations were made until completion of the 14-day observation period. Each animal was weighed daily from the day of arrival to completion of the study. At the end of the 14-day observation period, all surviving animals were killed and examined externally and internally for macroscopic abnormalities. Particular attention was paid to the frontal and nasal areas, the lungs and pleural surfaces. The lungs, liver and kidneys were weighed and the larynx, lungs, liver and kidneys were preserved. At the time of exposure the rats weighed 243 to 283 g for males and 213 to 247 g for females. They were 7 to 12 weeks old at this time and had been acclimatised for at least 5 days.	

5.2	**Results and discussion**	Dust atmosphere characterization (See Table A6.1.3.1-1) The airborne concentrations achieved were 0.259, 0.523 and 0.929 mg/l (determined gravimetrically). The percentage of particles with a diameter smaller than 6 µm were 40%, 45% and 29% of the low, mid and high test concentration, respectively. Mortality (See Table A6.1.3.1-2) 4-h inhalation exposure caused lethality at all three concentrations tested, all deaths occurring during the 48 hours immediately following exposure. Clinical signs During exposure, increased or decreased respiratory rate, struggling in the restraint tube and wet fur were observed. All observations were considered to be non-specific responses to exposure to a particulate atmosphere. During the three hours immediately following the exposure, hypothermia was recorded for all animals of 0.523 mg/l and some of the animals 0.259 mg/l. Other signs recorded during this period were hunched posture, tremors and vocalisation when handled. In addition, brown staining of the fur and wet fur were observed for most animals. Signs that persisted or that developed during Days 2 to 14 of the observation period included hunched posture, piloerection, penis mutilation, yellow staining, salivation, tremors, convulsions ataxia, hairloss and vocalisation when handled. Bodyweight All surviving animals lost, or gained a minimal amount of bodyweight during the first 24 hours after exposure. Surviving animals exposed to 0.929 mg/l lost weight for a further three days following exposure. Survivors exposed to 0.259 mg/l and 0.523 mg/l lost bodyweight or gained no weight for a further two days. Subsequently, the observed weight gain was within the expected range. Post mortem examination The external appearance at necropsy was consistent with the clinical observation of discolouration of the fur. There were no internal findings that were attributed to treatment. Organ weight analysis indicated that the lung weights of decedents were higher than expected. There were no other organ weight changes.	**X**
5.3	**Conclusion**	The acute (4-hour) inhalation LC_{50} of Fipronil to rats was 0.682 mg/l of air for male and female rats combined.	**X**
5.3.1	Reliability	1	
5.3.2	Deficiencies	The percentage of respirable particles of the tested dust aerosol was 29.7–45%. In a subsequent study, the test compound was air-milled to generate especially small particle sizes prior to exposure of rats (see XXXX, 1995; DocID XXXX; summarised under A6.1.3/02).	

Table A6.1.3.1-1 Acute inhalation study – atmosphere characterisation

Parameter	~~Nominal concentration (mg/l)~~		
Nominal concentration (mg/l)	2.1344	0.766	0.456
Achieved gravimetric concentration (mg/l)	0.929	0.523	0.259
Particle size (%<6µ)	29.7	45	40
Chamber temperature (°C)	20.7 ± 0.4	20.2 ±0.4	19.9 ±0.5
Chamber humidity (%)	58.8 ± 1.2	60.9 ±2.8	64.9 ±1.5

X

Table A6.1.3.1-2 Acute inhalation toxicity – mortality

Group	Achieved concentration (mg/l)	Mortality		
		Male	Female	Total
1	0.929	4/5	3/5	7/10
2	0.523	1/5	2/5	3/10
3	0.259	0/5	1/5	1/10
LC_{50} 0.682 mg/l				

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with the applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*as given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*as given in section 2*~~ none 3.1.2.3 Stability: ~~*stable*~~ The-stability was the responsibility of the sponsor. 3.3.15 Controls: ~~*Vehicle only*~~ exposed to filtered air 3.3.9 Particle size: *Group 1:* 0.929 mg/l: *29.7% (SD 8.2)* *Group 2:* 0.523 mg/l: *45.0% (SD 2.0)* *Group 3:* 0.259mg/l: *40.0% (SD 5.9)* 3.4 Examinations: Each rat was weighed daily from the day of delivery until the end of the observation period.
Results and discussion	Agree with the applicant's version.
Conclusion	Agree with the applicant's version. **Revisions/amendments:** 5.2 Results and discussion: ~~*29%*~~ 29.7% 5.3 Conclusion: *LC50 of Fipronil to rats was 0.632 mg/l of air* (0.426-0.938 mg/l)
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with air-milled Fipronil**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.3/02** XXXX. (1995) Fipronil: Acute nose-only dust inhalation toxicity study in rats. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 81-3 (1984) OECD 403 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**x**
3.1.1	Lot/Batch number	10MTD20	
3.1.2	Specification	As given in Section 2	**x**
3.1.2.1	Description	Fine white powder	
3.1.2.2	Purity	96.7%	
3.1.2.3	Stability	Stable	**x**
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley albino	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Approximately 47 days old 215 – 251 g (males)/ 182 – 220 g (females	
3.2.6	Number of animals per group	10 – 5 males and 5 females	
3.2.7	Control animals	No	**x**
3.3	**Administration/ Exposure**	Inhalation	
3.3.1	Post exposure period	14 days	
3.3.2 – 3.3.7		Not applicable (sections specific to acute oral toxicity studies)	
3.3.8	Concentrations	Nominal concentration 14.5, 5.4 and 5 mg/l Analytical concentration 0.72, 0.52 and 0.33mg/l	
3.3.9	Particle size	MMAD = 1.66 µm; GD = 0.3 µm; 97% of particles < 1.3 µm	**x**
3.3.10	Type or preparation of particles	The test substance was air-milled to generate a dust of exceptionally small mean particle size.	
3.3.11	Type of exposure	nose only	
3.3.12	Vehicle	None	

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with air-milled Fipronil**

3.3.13	Concentration in vehicle	n.a	
3.3.14	Duration of exposure	4 h	
3.3.15	Controls	Vehicle only	x
3.4	**Examinations**	All animals were individually observed for signs of toxic effects except during the exposure. During the exposure, observations were recorded approximately every 30min. Detailed individual examinations were performed just prior to and shortly following exposure, at approximately 1 and 2 hours following exposure, and once each day during the post exposure period. Body weight data were collected for all animals on the morning prior to initiation of exposure (denoted as Study Day 0) at 7 and 14 days following exposure. A complete necropsy was performed on all animals. The animals were anesthetized with methoxyflurane and killed by exsanguinations.	x
3.5	**Method of determination of LD_{50}**	Thompson	
3.6	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Mortality (see Table A6.1.3.2-2) All animals exposed to 0.52 and 0.72 mg/l died. At the low concentration of 0.33 mg/l, two deaths occurred in males only. The majority of deaths occurred within the first 48 hours following the end of treatment. Clinical signs (see Table A6.1.3.2-3) On the day of exposure (Day 0), the principal clinical signs seen at all dose levels were wetness (of the periocular, body and urogenital regions and occasionally of the perinasal area), unkempt fur and fine whole body tremors. Incidents of red encrustations of the periocular, perinasal and perioral regions were also seen in 2 females at the high dose level (0.72 mg/l) and in all rats at the low dose level, 0.33 mg/l. During the post-exposure period (Day 1 onwards), wetness, unkempt fur, hypoactivity and incoordination were the principal findings amongst survivors. Hypoactivity and incoordination were seen for up to three days. Hyperactivity was also noted at the high dose level (0.72 mg/l) in one male (on Day 4 only) and one female (on Days 2 to 4); both rats died on Days 8 and 12, respectively. The same male exhibited a swollen penis on Day 7. Closure of the left eye was seen on Day 1 in a single female exposed to 0.33 mg/l. All survivors at the low dose level of 0.33 mg/l were normal by Day 6.	x

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with air-milled Fipronil**

4.2	**Pathology**	Treatment-related necropsy findings for animals that died during the study included stained fur and/or encrustations of the perioral, perinasal, periocular and perineal areas. Other possible treatment-related changes in decedents were seen in the stomach and included discolouration (red or black foci in six rats), possible ulcerated area (in one rat) and a thickened white surface (in two animals). All other findings in decedents were considered to be unrelated to exposure to Fipronil. No treatment-related changes were observed in any survivors from the 0.33 mg/l group.
4.3	**Other**	Bodyweight A loss of bodyweight or decreased bodyweight gain was observed during the first week post exposure in survivors from the 0.33 and 0.72 mg/l group, the only dose groups with survivors. During the second week post exposure all rat in the 0.33 mg/l group gained weight.
4.4	**LD_{50}**	The results of this study indicate that a steep concentration-response curve exists for exposure to fipronil dust. The 4 hour LC_{50} values (with 95% confidence limits) were 0.36 (0.23 to 0.55) mg/l for males, 0.42 (0.34 to 0.51) mg/l for females and 0.39 (0.35 to 0.44) mg/l for the combined sexes.
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil was administered as a dust aerosol to rats for 4 hours by continuous snout-only exposure in order to assess the acute inhalation toxicity. The test compound was air-milled to generate especially small particle sizes and thus to increased the percentage of respirable particles. Three groups of 5 male and 5 female Sprague-Dawley rats were exposed by the nose-only continuously for 4 hours to nominal atmosphere concentrations of 5.0, 5.4 or 14.5 mg/litre of the test substance. Using an auger-type dust feed mechanism, the dust was mixed with a stream of compressed air, which carried it to the top of a glass chromatography tank, the mixing chamber, where non-respirable particles were removed. The dust aerosol was then fed into the inhalation chamber at an airflow rate of 8 l/min. After a 4-minute equilibration time (the theoretical time required for the dust aerosol to reach to 99% of the target atmosphere concentration), the 4-hour exposure period was initiated. Atmosphere measurements were the achieved gravimetric concentration, particle size distribution, chamber temperature and oxygen content. The relative humidity of the dry compressed air supply was also determined. Detailed examinations of each animal were performed just prior to exposure, at approximately 1 and 2 hours following exposure and at least once daily during the post exposure period. Additional daily checks were made for mortality. Individual bodyweights were recorded prior to exposure and at 7 and 14 days following exposure. All animals were examined at necropsy for gross lesions. The respiratory tract and all macroscopic lesions were preserved. On the day of exposure the rats weighed 215 to 251 g (males) and 182 to 220 g (females) and were approximately 47 days old. They were acclimatised for 5 days prior to treatment.

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with air-milled Fipronil**

5.2	**Results and discussion**	Achieved atmosphere characteristics (see Table A6.1.3.2-1) 97% of the dust particles had a diameter smaller than 3 µm. Mortality (see Table A6.1.3.2-2) All animals exposed to 0.52 and 0.72 mg/l died. At the low concentration of 0.33 mg/l, two deaths occurred in males only. The majority of deaths occurred within the first 48 hours following the end of treatment. Clinical signs (see Table A6.1.3.2-3) On the day of exposure (Day 0), the principal clinical signs seen at all dose levels were wetness (of the periocular, body and urogenital regions and occasionally of the perinasal area), unkempt fur and fine whole body tremors. Incidents of red encrustations of the periocular, perinasal and perioral regions were also seen in 2 females at the high dose level (0.72 mg/l) and in all rats at the low dose level, 0.33 mg/l. During the post-exposure period (Day 1 onwards), wetness, unkempt fur, hypoactivity and incoordination were the principal findings amongst survivors. Hypoactivity and incoordination were seen for up to three days. Hyperactivity was also noted at the high dose level (0.72 mg/l) in one male (on Day 4 only) and one female (on Days 2 to 4); both rats died on Days 8 and 12, respectively. The same male exhibited a swollen penis on Day 7. Closure of the left eye was seen on Day 1 in a single female exposed to 0.33 mg/l. All survivors at the low dose level of 0.33 mg/l were normal by Day 6. Bodyweight A loss of bodyweight or decreased bodyweight gain was observed during the first week post exposure in survivors from the 0.33 and 0.72 mg/l group, the only dose groups with survivors. During the second week post exposure all rat in the 0.33 mg/l group gained weight. Post mortem examination Treatment-related necropsy findings for animals that died during the study included stained fur and/or encrustations of the perioral, perinasal, periocular and perineal areas. Other possible treatment-related changes in decedents were seen in the stomach and included discolouration (red or black foci in six rats), possible ulcerated area (in one rat) and a thickened white surface (in two animals). All other findings in decedents were considered to be unrelated to exposure to Fipronil. No treatment-related changes were observed in any survivors from the 0.33 mg/l group.	x
5.3	**Conclusion**	The acute 4-hour LC_{50} (with 95% confidence limits) of air-milled Fipronil to rats was: Males: 0.36 (0.23 to 0.55) mg/l Females: 0.42 (0.34 to 0.51) mg/l Combined sexes: 0.39 (0.35 to 0.44) mg/l	
5.3.1	Reliability	1	

Section A6.1.3	**Acute inhalation toxicity**
Annex Point IIA, VI.6.1.3	**– with air-milled Fipronil**

5.3.2	Deficiencies	No

Table A6.1.3.2-1. Atmosphere Characteristics

Parameter	**~~Nominal concentration (mg/l)~~**		
Nominal concentration (mg/l)	14.5	5.4	5.0
Achieved gravimetric concentration (mg/l	0.72	0.52	0.33
Chamber temperature (°C)	23±0	21±0	22±0
Chamber humidity (%)	26±1	19±3	24±4
Oxygen content	Approx 20.4%		
MMAD	1.66µm		
Geometric standard deviation	1.3µm		
Respirable particles (% <3 µm)	97		

X

Table A6.1.3.2-2 Acute inhalation toxicity – mortality

Group	**Achieved concentration (mg/l)**	**Mortality**		
		Male	**Female**	**Total**
3	0.33	2/5	0/5	2/10
2	0.52	5/5	5/5	10/10
1	0.72	5/5	5/5	10/10

Table A6.1.3.2-3 Acute inhalation toxicity – clinical signs

Time	**Clinical sign**	**Dose level**					
		0.33 mg/l		**0.52 mg/l**		**0.72 mg/l**	
		M	**F**	**M**	**F**	**M**	**F**
Day 0 (day of exposure)	Wetness	5/5	5/5	5/5	5/5	5/5	5/5
	Unkempt fur	5/5	5/5	5/5	5/5	5/5	5/5
	Tremors	5/5	5/5	5/5	5/5	5/5	5/5
	Encrustations	5/5	5/5	0/5	0/5	0/5	~~2/5~~ 0/5
Days 1 -14 (post exposure)	Wetness	4/5	5/5	0/5	0/5	1/5	1/5
	Unkempt fur	4/5	5/5	~~0/5~~ 1/5	2/5	1/5	~~1/5~~ 3/5
	Encrustations	4/5	5/5	~~0/5~~ 1/5	2/5	1/5	~~1/5~~ 3/5
	Hypoactivity	4/5	1/5	0/5	2/5	1/5	1/5
	Incoordination	4/5	5/5	0/5	0/5	1/5	1/5
	Hyperactivity	0/5	0/5	0/5	0/5	1/5	1/5
	Swollen penis	0/5	0/5	0/5	0/5	1/5	0/5
	Closed left eye	0/5	1/5	0/5	0/5	0/5	0/5

X

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*as given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*as given in section 2*~~ none 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor. 3.2.7 Control animals: ~~*No*~~ historic controls 3.3.9 Particle size: ~~*GD = 0.3 µm; 97% of particles < 1.3 µm*~~ geometric standard deviation for the particle size determinations was approximately 1.3; 97% of particles ≤ 3 µm 3.3.15 Controls: ~~*Vehicle only*~~ 3.4 Examinations: Animals were checked once a day for mortality.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Clinical signs/5.2 Results and discussion: *Incidents of red encrustations of the periocular, perinasal and perioral regions were also seen in ~~2 females at the high dose level (0.72 mg/l) and in~~ all rats at the low dose level, 0.33 mg/l at Day0.* *During the post-exposure period (Day 1 onwards), wetness, unkempt fur,* red encrustation of the periocular, perinasal and perioral regions; *hypoactivity and incoordination were the principal findings amongst survivors. Hypoactivity and incoordination were ~~seen~~* in survivors from the 0.33 mg/l group *for up to three days* following exposure.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.4 **Annex Point IIA, VI6.1.4**	**Acute skin and eye irritation** **– Eye irritation in the rabbit**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.4/02** XXXX. MB 46030 (technical): Ocular irritancy study in the rabbit XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes 92/69/EEC A V B.5 OECD 405 EPA 81-4	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	78/GC/90	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	White powder	
3.1.2.2	Purity	96.7%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	XXXX	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Approximately 12 to 18 weeks Males: 3.1 to 3.2 kg Females: 2.4.to 2.5 kg	
3.2.6	Number of animals per group	3 males and 3 females	
3.2.7	Control animals	No (The contralateral eye remained untreated and served as a control)	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	Test substance was used as delivered	
3.3.2	Amount of active substance instilled	90 mg equivalent to 0.1 ml of fipronil	
3.3.3	Exposure period	-	
3.3.4	Post exposure period	7 days	X
3.4	**Examinations**		
3.4.1	Opthalmoscopic examination	yes	
3.4.1.1	Scoring system	Draize	

Section A6.1.4 **Annex Point IIA, VI6.1.4**	**Acute skin and eye irritation** **– Eye irritation in the rabbit**	
3.4.1.2 Examination time points	60min, 1, 2, 3, 7, 10 and 14 days after instillation	
3.4.2 Other investigations	None	
3.5 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Clinical signs	None that would be regarded to indicate systemic toxicity	
4.2 Average score		
4.2.1 Cornea opacity	Average score (24h-48h-72h): 0	
4.2.2 Iris	Average score (24h-48h-72h): 0	
4.2.3 Conjunctiva		
4.2.3.1 Redness	Average score (24h-48h-72h): 0.78	
4.2.3.2 Chemosis	Average score (24h-48h-72h): 0.56	
4.3 Reversibility	Yes	
4.4 Other		
4.5 Overall result	Not an eye irritant. For details see Table A6.1.4.2-1	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fipronil was administered to the eyes of three male and three female New Zealand White rabbits. A weight (mean 90 mg) equivalent to a volume of 0.1 ml of Fipronil was placed into the lower everted lid (conjunctival sac) of one eye of each animal. The contralateral eye was untreated and served as a control. The eyes were not rinsed after treatment with the solid test substance. Rabbits were observed daily for clinical signs and mortality. The eyes were examined 1 hour after installation and after 1, 2, 3, 7, 10 and 14 days. All animals were killed on day 14.	
5.2 Results and discussion	(See Table A6.1.4.2-1) Ocular treatment caused minor transient corneal opacity in 2 out of 6 rabbits and iritis in 5 animals at 1-hour post-instillation. Both findings had resolved within 24 hours. Grade-1 to grade-2 conjunctival redness was also seen in all six rabbits within 1 hour of instillation, which was associated with slight swelling and discharge in four cases. The eyes of one rabbit were normal at 48 hours and in a further two by 72 hours. In the remaining three rabbits, minor conjunctival redness was reversible within 14 days.	**x**
5.3 Conclusion	Under the conditions of this study, Fipronil is not an eye irritant according to EU or GHS classification criteria.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Table A6.1.4.2-1 Results of eye irritation study

Ocular reactions	Score Time point	Rabbit No.						Mean irritation score (24-h – 48-h – 72-h)
		1	2	3	4	5	6	
Cornea opacity	1-h	0	0	1 [a2]	1 [a4]	0	0	
	24-h	0	0	0	0	0	0	0.00
	48-h	0	0	0	0	0	0	
	72-h	0	0	0	0	0	0	
	7-14d	0	0	0	0	0	0	
Iris	1-h	0	1	1	1	1	1	
	24-h	0	0	0	0	0	0	0.00
	48-h	0	0	0	0	0	0	
	72-h	0	0	0	0	0	0	
	7-14d	0	0	0	0	0	0	
Conjuctival redness	1-h	1	2	2	2	2	2	
	24-h	1	1	1	1	1	1	0.78
	48-h	1	1	1	1	0	1	
	72-h	0	1	0	1	0	1	
	7-d	0	1	0	1	0	1	
	10-d	0	1	0	1	0	1	
	14-d	0	0	0	0	0	0	
Conjunctival chemosis	1-h	0 [d0]	1 [d1]	1 [d1]	1 [d1]	1 [d1]	1 [d1]	
	24-h	0 [d0]	1 [d1]	1 [d1]	1 [d1]	1 [d1]	1 [d1]	0.56
	48-h	0 [d0]	1 [d1]	0 [d0]	1 [d1]	0 [d0]	1 [d1]	
	72-h	0 [d0]	1 [d1]	0 [d0]	1 [d1]	0 [d0]	0 [d0]	
	7-d	0 [d0]	0 [d0]	0 [d0]	~~1~~ [d1] 0	0 [d0]	0 [d0]	
	10-14d	0 [d0]	0 [d0]	0 [d0]	0 ~~[d1]~~ [d0]	0 [d0]	0 [d0]	

X

a2 (a4) = Corneal opacity area score 2 (4); d0 (d1) = Conjunctival discharge score 0 (1)

Table A6.1.4.1-1 Results of skin irritation study in rabbits

Rabbit number	Sex	Skin response	Time post exposure					Mean irritation score
			1 hr	24-hr	48-hr	72-hr	7 days	
92-12979	Male	Erythema	1	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
93-1060	Male	Erythema	1	1	1	1	0	1.0
		Oedema	0	0	0	0	0	0
93-327	Male	Erythema	1	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
92-12518	Female	Erythema	1	0	0	0	0	0
		Oedema	0	0	0	0	0	0
93-116	Female	Erythema	2	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
93-346	Female	Erythema	1	0	0	0	0	0
		Oedema	0	0	0	0	0	0
Mean Erythema (24–48–72-h) score				**0.33**				
Mean Oedema (24–48–72-h) score				**0.00**				

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: XXXX 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor 3.3.4 Post exposure period: ~~*7 days*~~ 14 days
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *with slight swelling and discharge* ~~*in four cases*~~ in five cases
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	Not take into account the table A6.1.4.1-1 which represents the results of skin irritation study in rabbits.
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.4 **Annex Point IIA, VI.6.1.4**	**Acute skin and eye irritation** **– Skin irritation in the rabbit**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.4/01** XXXX. MB 46030 (technical): cutaneous irritancy study in the rabbit. XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes 92/69/EEC A V B.4 OECD 404 EPA 81-5	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	Batch: 78/GC/90	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	White powder	
3.1.2.2	Purity	96.7%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and females	
3.2.5	Age/weight at study initiation	12 – 18 weeks of age Males: 3.1 to 3.4 kg Females: 3.2 to 3.3 kg	
3.2.6	Number of animals per group	3 males and 3 females	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Pre-exposure period		
3.3.1.1	Preparation of test substance	0.5 g of test substance was moistened with 0.5 ml of corn oil.	
3.3.1.2	Test site and preparation of test site	Dorso-lumber area of the trunk Application site skin was clipped/trimmed free of air up to 1 day before substance application.	X
3.3.2	Occlusion	Semi occlusive	
3.3.3	Vehicle	none	X
3.3.4	Concentration in vehicle	n.a.	X
3.3.5	Total volume applied	0.5 g	X

Section A6.1.4 **Annex Point IIA, VI.6.1.4**	**Acute skin and eye irritation** **– Skin irritation in the rabbit**

3.3.6 Removal of test substance	Water	x
3.3.7 Duration of exposure	4 hours	
3.3.8 Post exposure period	7 days	
3.3.9 Controls	None	
3.4 Examinations		
3.4.1 Clinical signs	Yes	
3.4.2 Dermal examination	Yes	
3.4.2.1 Scoring system	Draize (1959)	
3.4.2.2 Examination time points	1 h, 24 h, 48 h, 72 h, 7 days	
3.4.3 Other examinations	None	
3.5 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Average score		
4.1.1 Erythema	0.33 (24-48-72h)	
4.1.2 Oedema	0.0 (24-48-72h)	
4.2 Reversibility	Yes within 7 days	
4.3 Other examinations	n.a.	
4.4 Overall result	Not irritating to skin	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	A single dose of 0.5 g of Fipronil, moistened with 0.5 ml of corn oil, was applied for 4 hours to the intact, clipped dorsal skin of three male and three female New Zealand White rabbits in order to assess the skin irritation potential. The dressing was removed at the end of the treatment period and skin responses assessed over 7 days post application. Rabbits were observed daily for clinical signs and mortality. The treated skin was examined approx. 1 hour after removal of the patches and on days 1, 2, 3 and 7 post application. All rabbits were sacrificed 7 days after treatment. The rabbits were approximately 12 to 18 weeks of age and weighed 3.1 to 3.4 kg on the day of treatment.	
5.2 Results and discussion	(See Table A6.1.4.1-1) One hour after removal of the dressing, one rabbit showed well-defined erythema (Grade 2), while in the other five rabbits, reddening of the skin was only barely perceptible (Grade 1). Very slight oedema (Grade 1) of the application skin was encountered in three rabbits, the other three rabbits did not show any signs of oedema. At the first time point relevant for scoring, after 24 hours, two of six rabbits were without any skin reaction, while the other four rabbits showed very slight (i.e. barely perceptible) reddening of the skin, which was reversible within 48 h for three rabbits and reversible within 7 days for one rabbit. The mean irritation score of 0.33 was obtained for erythema, and 0.0 for oedema.	
5.3 Conclusion	Under the conditions of this study, Fipronil is not a skin irritant according to EU classification criteria**.**	

Section A6.1.4 **Annex Point IIA, VI.6.1.4**	**Acute skin and eye irritation** **– Skin irritation in the rabbit**

5.3.1 Reliability 5.3.2 Deficiencies	1 No	

Table A6.1.4.1-1 Results of skin irritation study in rabbits

Rabbit number	**Sex**	**Skin response**	**Time post exposure**					**Mean irritation score**
			1 hr	**24-hr**	**48-hr**	**72-hr**	**7 days**	
92-12979	Male	Erythema	1	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
93-1060	Male	Erythema	1	1	1	1	0	1.0
		Oedema	0	0	0	0	0	0
93-327	Male	Erythema	1	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
92-12518	Female	Erythema	1	0	0	0	0	0
		Oedema	0	0	0	0	0	0
93-116	Female	Erythema	2	1	0	0	0	0.33
		Oedema	1	0	0	0	0	0
93-346	Female	Erythema	1	0	0	0	0	0
		Oedema	0	0	0	0	0	0
Mean Erythema (24–48–72-h) score				**0.33**				
Mean Oedema (24–48–72-h) score				**0.00**				

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: Report No: XXXX 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor 3.3.1.2 Test site and preparation of test site: ~~*Dorso-lumber area of the trunk*~~ dorsal area of the trunk 3.3.3 Vehicle: ~~*none*~~ corn oil 3.3.4 Concentration in vehicle: ~~*n.a.*~~ 1 g/ml 3.3.5 Total volume applied: ~~*0.5 g*~~ not submitted 3.3.6 Removal of test substance: *water* or other appropriate solvent.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.5/01** XXXX M&B 46030: Delayed contact hypersensitivity study in Guinea-pigs. XXXX (unpublished) (XXXX) **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 15 March 2007 XXXX (unpublished)	**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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 406 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Fine off-white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	**X**
3.1.2.4	Preparation of test substance for application	<u>for induction:</u> propylene glycol/Freunds Complete Adjuvant <u>for challenge:</u> propylene glycol	**X**
3.1.2.5	Pretest performed on irritant effects	Yes	
3.2	**Test Animals**		

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**	
3.2.1 Species	Guinea pigs	
3.2.2 Strain	Dunkin-Hartley	
3.2.3 Source	XXXX	**x**
3.2.4 Sex	Male and Female	
3.2.5 Age/weight at study initiation	Age : 6 – 8 weeks 350 – 437 g	**x**
3.2.6 Number of animals per group	10	
3.2.7 Control animals	Yes	**x**
3.3 Administration/ Exposure	Adjuvant study	
3.3.1 Induction schedule	Day 1 – day –8 – day 22 (See Table A6.1.5.1-1)	**x**
3.3.2 Way of induction	Intradermal and topical occlusive	**x**
3.3.3 Concentrations used for induction	5% w/v	
3.3.4 Concentration Freunds Complete Adjuvant (FCA)	50%	
3.3.5 Challenge schedule	day 22 (See Table A6.1.5.1-1)	**x**
3.3.6 Concentrations used for challenge	3 and 10% w/v	
3.3.7 Rechallenge	No	
3.3.8 Scoring schedule	24 and 48 hours (See Table A6.1.5.1-1)	
3.3.9 Removal of test substance	bandage removed after 24 hours	
3.3.10 Positive control substance	Yes 10% and 5% w/v Benzocaine in propylene glycol (separate experiment carried out at the test facility from 11 Nov 1992 to 5 December 1992); see XXXX. 2007 (6.1.5/03)	
3.4 Examinations		
3.4.1 Pilot study	Yes	**x**
3.5 Further remarks		**x**

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**

		4. RESULTS AND DISCUSSION			
4.1	**Results of pilot studies**	Pre-treatment formulation trials indicated that 30% w/v Fipronil in propylene glycol (pg) or adjuvant (FCA) was the maximum concentration that would pass through a hypodermic needle. The maximum practicable concentration of the test material in the chosen vehicle for topical administration was 50% w/v. The following irritation screen tests were performed: (1) Intradermal application of 30, 10, 5, 3, 1, 0.5% w/v in pg or FCA resulted in moderate erythema (grade 2) in the animals tested at 24 and 48 hours, reversible within 7 days. (2) At least five days after intradermal treatment with FCA, two guinea pigs were subsequently topically exposed for 48 hours to 50, 30, 10 and 5% w/v fipronil in pg under occlusive patch. The guinea pigs showed barely perceptible erythema at the site treated with 50% w/v fipronil 24 h after removal of the dressing. Both guinea pigs were difficult to handle and were irritable and overactive 24 h after bandage removal. Both guinea pigs were found dead after 48 hours. (3) At least 20 days after intradermal treatment with FCA, three guinea pigs were topically exposed for 24 hours to 50, 30, 10 and 5% w/v fipronil in pg under occlusive patch. The guinea pigs showed slight or barely perceptible erythema at the site treated with 50% w/v or 30% w/v fipronil after 24 h and/or 48 h. All three guinea pigs showed nervousness and slight tremor 24 and 48 hours after bandage removal.			**x**
4.2	**Results of test**				
4.2.1	24 h after challenge	<u>Vehicle treatment</u> Neg. control: 1/19 Test substance: 0/20	<u>3% fipronil in propylene glycol:</u> Neg. control: 0/19 Test substance: 0/20	<u>10% fipronil in propylene glycol:</u> Neg. control: 0/19 Test substance: 4/20	**x**
4.2.2	48 h after challenge	<u>Vehicle treatment</u> Neg. control: 0/20 Test substance: 0/20	<u>3% fipronil in propylene glycol:</u> Neg. control: 0/19 Test substance: 0/20	<u>10% fipronil in propylene glycol:</u> Neg. control: 0/19 Test substance: 2/20	**x**
4.2.3	Other findings	During the induction applications, test animals were agitated and overactive on the second, third and fourth days after the intradermal injections. No systemic reactions were observed following topical application but animals were overactive and/or agitated when clipped or shaved prior to treatment. All animals achieved the anticipated overall bodyweight gains.			**x**
4.3	**Overall result**	Fipronil is not a skin sensitizer			

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Fipronil was administered intradermally and topically to guinea pigs according the Magnusson & Kligman Maximisation Test in order to assess its skin sensitisation potential. Groups of ten male and ten female Dunkin-Hartley guinea pigs were allocated to one test and one control group. A further five males and four females were used for preliminary dose range finding to identify concentrations that were a) well tolerated systemically by intradermal injection and topical application for the induction phases and b) the maximum non-irritant concentration for the topical challenge. The guinea pigs were six to eight weeks old on Day 1 and weighed 350 – 437 g (controls) and 355 – 418 g (test animals). They were acclimatised for between six and sixteen days prior to the first administration of the test material. Induction phase: On Day 1, three intradermal injections were made into the closely clipped dorsal skin of ten male and ten female guinea pigs which were covered by an occlusive dressing for 48 hours. These intradermal injections comprised: Freunds Complete Adjuvant (FCA) in purified water, 1:1 5% w/v Fipronil in propylene glycol 5% w/v Fipronil in propylene glycol in FCA Seven days later (on Day 8) the same area of skin was treated with a topical application of 5% Fipronil in propylene glycol and the test site was covered by an occlusive dressing for 48 hours. The same induction procedures were carried out on the control group, except that the test material was replaced by the vehicle. Treated skin sites were observed for any reaction 24 hours and 48 hours after injection or removal of the occlusive dressing. Challenge phase: On Day 22, single topical challenge applications of 3% and 10% w/v of Fipronil in propylene glycol were applied to separate areas of the shaved right flank of all animals. The vehicle alone was applied to the shaved left flank. All sites were occluded for 24 hours. The dressing was removed the following day and the skin sites observed approximately 24 and 48 hours later. Positive control group experiment: The study design was similar to that described above. 20 guinea pigs received intradermal induction treatment with 3% benzocaine in propylene glycol or in in propylene glycol and FCA, followed by topical induction treatment with 30% benzocaine. The control group consisting of 10 guinea pigs received the vehicle without benzocaine. Challenge treatment of treatment and control group animals was performed with 10% or 5% benzocaine in propylene glycol.	x

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**	
	Evaluation criteria for skin responses: A significant erythematous reaction was considered to be Grade 1 erythema or above. Barely perceptible erythema was considered not to be a significant response or a conclusive indication of delayed contact hypersensitivity since this is often a non-specific response to the dosing procedure. The incidence of significant erythematous reactions was scored in the test and control group. According to EU evaluation criteria, an incidence of at least 30% of the test animals was considered to be a positive indication of delayed contact hypersensitivity. Other observations Animals were observed daily for mortality and clinical signs. Individual bodyweight was recorded at weekly intervals. All guinea pigs were killed at termination.	
5.2 Results and discussion	Clinical signs and mortality There were no substance-related deaths. One control animal had in-depth damage to the anterior intradermal injection site on Day 13 and was killed on humane grounds. All surviving animals remained in overt good health. During the induction applications, test animals were agitated and overactive on the second, third and fourth days after the intradermal injections. No systemic reactions were observed following topical application but animals were overactive and/or agitated when clipped or shaved prior to treatment. All animals achieved the anticipated overall bodyweight gains. Skin responses during the induction phase: Intradermal injection of 5% w/v Fipronil in FCA caused slight or moderate erythema, pallor and discolouration of the skin and, in two cases, eschar. However, intradermal injection of 5% w/v Fipronil in propylene glycol produced no dermal reaction. No dermal reaction was seen after topical application of 5% w/v Fipronil in propylene glycol.	
	Skin responses during the challenge phase: (See Table A6.1.5.1-2) A significant dermal response (slight erythema or eschar formation) was observed in four test guinea pigs after challenge with 10% w/v Fipronil in propylene glycol. No responses were seen in control animals similarly challenged. No significant skin response was observed after challenge with 3% w/v Fipronil in propylene glycol. One control exhibited a significant reaction. Although the incidence and severity of response in the test group challenged with 10% w/v Fipronil were slightly greater than in controls, the reactions were largely confined to the 24-hour examination. The total number of test animals with a positive response was less than the EU limit for classification (30%).	**X**
	Results of positive control experiment: (See Table A6.1.5.1-2) Challenge treatment with 10% w/v benzocaine in propylene glycol gave skin responses in 12/20 animals compared to 1/10 in the control group. The experiment demonstrated that the test system was able to reliably identify skin sensitisers.	**X**

Section A6.1.5 **Annex Point IIA, VI.6.1.5**	**Skin sensitisation**

5.3 Conclusion	Fipronil did not cause delayed contact hypersensitivity, i.e., was not a skin sensitizer, in this Magnusson and Kligman Maximisation test in guinea pigs according to EU Classification criteria.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	No	

Table ~~A6.1.5-1~~ A6.1.5.1-1 Detailed information including induction/challenge/scoring schedule for skin sensitisation test

X

Treatment phase	**Magnusson-Kligman test**		**Observations/Remarks**
	Day of treatment	**Application**	
Induction 1	1	intradermal	Animals administered 5% fipronil were agitated and overactive on days 2, 3, 4~~, 7 and 8~~ (on day 8 during shaving)
Induction 2	8–10	topical	No dermal or systemic reactions after treatment
Challenge	22–23	topical	No systemic effects reported
Scoring 1	24	–	–
Scoring 2	25	–	–

X

Table ~~A6.1.5-2~~ A6.1.5.1-2 Result of skin sensitisation test

X

Group	**Treatment**	**Number of animals**	**Number of animals with significant response[+]**		**Total number of animals with a positive response**
			24 hours	**48 hours**	
Control	Propylene glycol	19[#]	1	0	1
Test	Propylene glycol	20	0	0	0

Group	Treatment	Number of animals	Number of animals with significant response[+]		Total number of animals with a positive response	
			24 hours	48 hours		
Control	3% w/v Fipronil in propylene glycol	19	0	0	0	
Test	3% w/v Fipronil in propylene glycol	20	0	0	0	
Control	10% w/v Fipronil in propylene glycol	19	0	0	0	
Test	10% w/v Fipronil in propylene glycol	20	4	2	4	
Positive control experiment in Dunkin Hartley guinea pigs- <u>Dermal sensitization responses to challenge Benzocaine</u> (XXXX., 11 November - 5 December 1992) data from XXXX. 2007 (6.1.5-3; XXXX)						X
~~Positive~~ <u>control group</u>	5% w/v Benzocaine in propylene glycol	10	0	0	0	X
~~Control~~ <u>Test group</u>	5% w/v Benzocaine in propylene glycol	19	3	1	4	X
~~Positive~~ <u>control group</u>	10% w/v Benzocaine in propylene glycol	10	1	0	1	X
~~Control~~ <u>Test group</u>	10% w/v Benzocaine in propylene glycol	19	10	3	12	X

[+] Slight erythema or a more marked response (Grade 1 or above); [#] One animal humanely killed on Day 13

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *XXXX* 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor 3.1.2.4 Preparation of test substance for application: Freunds Complete Adjuvant was prepared immediately before use by emulsifying equal volumes of purified water and the concentrate of the complete adjuvant. 3.2.3 Source: XXXX 3.2.5 Age/weight at study initiation: ~~*350-437 g*~~ 355-418 g 3.2.7 Control animals: *yes* The control animals were treated identically to the test animals during the induction and the challenge procedures, except that during induction the test material was replaced by vehicle. 3.3.1 Induction schedule: *Day 1:* 3 pairs of injections (0.1 ml) were made deep into the dermis, such that on either side of the dorsal median line there were 3 injection sites in a row parallel to the spinal column. *Day 8:* The dermal site overlying the scapulae was treated by topical application of 0.6 ml of the test material formulation to test animals. Each dose was applied to the skin and covered by an occlusive dressing for 48 hours. The application site was wiped with a paper tissue moistened with the vehicle immediately after removal of the bandage. ~~*Day 22*~~ 3.3.2 Way of induction: *Intradermal and topical* (occlusive dressings) 3.3.5 Challenge schedule: Both flanks of all animals were clipped on day 21 to expose areas on either side of the trunk. On day 22 these areas were wet shaven . Approximately 3 hours later the left side was treated by topical application of 0.03 ml of the vehicle while the right side received 0.03 ml of the maximum non-irritant concentration to one site and a dilution to a second site. The doses were covered by an occlusive dressing for 24 hours. The test site was wiped with a paper tissue moistened with vehicle immediately after removal of the bandage. 3.4.1 Pilot study: The study design included a primary skin irritation screen which imposed limits on the concentration of test material used during the main study. 3.5 Further remarks: Animals were observed daily for mortality and clinical

	signs. Individual bodyweight was recorded at weekly intervals. All guinea pigs were killed at termination.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Results of pilot studies: *Intradermal application of 30, 10, 5, 3, 1, 0.5% w/v in pg or FCA resulted in moderate erythema (grade 2) in the animals tested at 24 and 48 hours* after injection , *reversible within 7 days* .One animal showed convulsions 6 days after injection and was found dead on day 7. Another animal showed prone, gasping, pallor and tremor. Agitation and tremor were observed in two other guinea-pigs ,7 days after injection. ~~*The guinea pigs*~~ One guinea pig *showed barely perceptible erythema at the site treated with 50% w/v fipronil 24 h after removal of the dressing.* 4.2.1 24 h after challenge: *0/19** *one control animal was humanely killed on day 13 because it was found with in-depth damage to the anterior intradermal injection site. 4.2.2 48 h after challenge: *Vehicle treatment: Neg. control: ~~0/20~~* 0/19 4.2.3 Other findings: Skin responses during the induction phase: Intradermal injection of 5% w/v Fipronil in FCA caused slight or moderate erythema, pallor and discolouration of the skin and, in two cases, eschar. However, intradermal injection of 5% w/v Fipronil in propylene glycol produced no dermal reaction. No dermal reaction was seen after topical application of 5% w/v Fipronil in propylene glycol.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *On Day 1, three* pairs of *intradermal injections were made into the closely clipped dorsal skin of ten male and ten female guinea pigs ~~which were covered by an occlusive dressing for 48 hours.~~* 5.2 Results and discussion: *One control exhibited a significant reaction* with a topical application of propylene glycol alone *Results of positive control experiment: Challenge treatment with 10% w/v benzocaine in propylene glycol gave skin responses in 12~~/20~~* 19 *animals compared to 1/10 in the control group.*
Reliability	1
Acceptability	acceptable
Remarks	A dose of 0.5% of fipronil for intradermal injection would have been sufficient because this dose induced a moderate erythema as the dose finally retained for the main test's injection (5% of firponil).
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.1.5/02** XXXX M&B 46030: Dermal sensitization study in guinea-pigs. XXXX GLP (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 406 (1981) US EPA 81-6	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Fine off-white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	**X**
3.1.2.4	Preparation of test substance for application	<u>for induction:</u> paraffin oil <u>for challenge:</u> paraffin oil	
3.1.2.5	Pretest performed on irritant effects	Yes: 4 animals received 25 ml of 4 concentrations(3%, 5%, 10%, 30% w/v) on the wet-shaven flanks (at 4 different sites) each under a 2x2 cm^2 patch and occlusive dressing, exposure duration was 6 hours. The top concentration of 30% was selected as the highest concentration that was considered practical for topical application. After treatment of the skin sites with depilation cream, the skin sites were assessed about 24 hours and 48 hours after treatment	**X**
3.2	**Test Animals**		
3.2.1	Species	Guinea pigs	
3.2.2	Strain	Dunkin-Hartley	

3.2.3	Source	XXXX	x
3.2.4	Sex	Male and Female	
3.2.5	Age/weight at study initiation	Body weight before treatment on day 1: 321 - 449 g Age :6–8 weeks (estimated based on body weight)	
3.2.6	Number of animals per group	Topical irritation screen: 4 animals (each animal treated with 4 concentrations at different test sites) Main test: 20 animals in test group (10/sex) 20 animals in vehicle control group (10/sex) 10 animals in positive control group (5/sex)	
3.2.7	Control animals	Vehicle control (paraffin oil) Positive control (dinitrochlorobenzene, DNCB)	
3.3	**Administration/ Exposure**		
3.3.1	Induction schedule	Days 1, 8 and 15	
3.3.2	Way of induction	Topical occlusive exposure under occlusive conditions for 6 hours	
3.3.3	Concentrations used for induction	Fipronil: 30% w/v in paraffin oil Positive control (DNCB): 3% w/v in absolute ethanol	
3.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not applicable in the Buehler test	
3.3.5	Challenge schedule	Day 29	
3.3.6	Concentrations used for challenge	Fipronil: 5 and 30% w/v Positive control (DNCB): 0.1% w/v in acetone	
3.3.7	Rechallenge	No	
3.3.8	Scoring schedule	Appox. 24 and 48 hours after challenge treatment	
3.3.9	Removal of test substance	Excess of dose material was washed with paraffin oil immediately after bandage removal	
3.3.10	Positive control substance	3% w/v of dinitrochlorbenzene	
3.4	**Examinations**		
3.4.1	Pilot study	Yes	
3.5	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Results of pilot studies**	The test substance proved to be non-irritant during a preliminary test (4 other animals) performed with fipronil in paraffin oil (range 3, 5, 10 and 30 % w/v).	

4.2	**Results of test**		
4.2.1	24 h after challenge	5% fipronil in paraffin oil: Neg. control group: 0/20 Test substance group: 0/20 0.1% DNCB in acetone: Neg. control group: 0/10 Test group group: 4/10	30% fipronil in paraffin oil Neg. control group: 0/20 Test substance group: 0/20
4.2.2	48 h after challenge	5% fipronil in paraffin oil: Neg. control group: 0/20 Test substance group: 0/20 0.1% DNCB in acetone: Neg. control group: 0/10 Test group group: 2/10	30% fipronil in paraffin oil Neg. control group: 0/20 Test substance group: 0/20
4.2.3	Other findings	There were no clinical signs of toxicity and no diffeence in bodyweight gain between treated and control animals.	
4.3	**Overall result**	Fipronil is not a skin sensitizer	

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Induction:
The shaved left flanks of 10 male and 10 female albino guinea-pigs (Durkin Hartley strain from Harlan Olac, Ltd., UK) were subjected to six-hour occluded topical applications of 30 % w/v fipronil in paraffin oil on Days 1, 8 and 15. A similar control group of guinea-pigs remained untreated during the induction phase of the study. The concentration used for topical induction was selected on the basis of a pretest, where 30% w/v was considered to be the highest applicable concentration in paraffin oil. A positive control group was exposed to 3% DNCB w/v in absolute ethanol for 6 hours under the same experimental conditions.

Challenge
On Day 29, all test and control animals were challenged by six-hour occluded topical applications of 30 % and 5 % w/w fipronil in paraffin oil to two sites on the right flank. Dermal responses to the challenge procedure were assessed approximately 24 and 48 hours after application. The positive control group was treated following the same procedures with 0.1 % w/v DNCB in acetone.

All animals were observed daily for clinical signs and mortality.
Individual bodyweights were recorded at weekly intervals.
All guinea pigs were killed at termination.

5.2	**Results and discussion**	Induction phase: Applications with 30% w/v Fipronil in paraffin oil did not cause any skin reaction. Challenge phase: Challenge application of 30% w/v Fipronil in paraffin oil caused grade ± ("very faint") erythema in one test and five control animals. Challenge application of 5% Fipronil in paraffin oil grade ± ("very faint") erythema in four test and five control animals. There were no clinical signs of toxicity and no difference in bodyweight gain between treated and control animals. In the positive control group induced with 3% DNCB, challenge application with 0.1% DNCB resulted in grade 1 ("faint") erythema in 4 of 10 test group animals, compared to 0 of 10 control group guinea pigs. Four further test group animals and one irritation control group guinea pig showed grade ± ("very faint") erythema. Discussion: Based on these findings, no significant skin reactions were observed that could be related to skin sensitisation potential of fipronil. The results obtained in the positive test group animals demonstrated that the system was capable of reliably detecting skin sensitisers.	
5.3	**Conclusion**	Fipronil did not cause delayed contact hypersensitivity, i.e., was not a skin sensitizer in this Buehler test in guinea pigs according to EU classification criteria.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: ~~*2 November 1990*~~ 17 September 1990 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: ~~*stable*~~ No analyses were undertaken to assess the stability, homogeneity or achieved concentrations of the test material in the vehicle. 3.1.2.5 Pretest performed on irritant effects: *4 animals received ~~25 ml~~ 0.25 ml of 4 concentrations* 3.2.3 Source: *XXXX*
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	The induction dose is the highest concentration which induces no severe irritation. However the concentration used in the induction phase does not induce irritation at all. It is the limit of the test.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.2 **Annex Point IIA, VI.6.2**	**Absorption, distribution, metabolism and excretion of ^{14}C-Fipronil in the rat after oral administration**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A6.2/01** XXXX (^{14}-C) M&B 46030: Absorption, distribution, metabolism and excretion in the rat. XXXX (unpublished) (XXXX) **A6.2/02** XXXX. Addendum to Report (^{14}-C) M&B 46030: Absorption, distribution, metabolism and excretion in the rat. XXXX (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 1.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: EU 94/79/EEC USEPA 85-1 OECD 417 (1984) JMAFF NohSan no 4200 (1985)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	Labelled fipronil : IHR 1465 Non labelled AJK 232/C2	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description		**X**
3.1.2.2	Purity	Labelled fipronil: 99.5% (lot 1) and 98.6% (lot 2) Non labelled: 99.4%	**X**
3.1.2.3	Stability	stable	**X**
3.1.2.4	Radiolabelling	^{14}C (phenyl ring uniformly labeled)	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Charles River Crl:CD	**X**
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	11 Weeks 173 – 304 g	**X**
3.2.6	Number of animals per group	10 (5 male and 5 female)	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Oral	

Section A6.2 **Annex Point IIA, VI.6.2**	**Absorption, distribution, metabolism and excretion of ^{14}C-Fipronil in the rat after oral administration**	
3.3.1 Type	Gavage	
3.3.2 Dose levels	Radiolabelled low dose: 4 mg/kg bw Radiolabelled high dose: 150 mg/kg bw	
3.3.3 Preparation of test substance	Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80	x
3.3.4 Total volume applied	5ml/kg bodyweight (nominal)	
3.3.5 Concentration in vehicle	Radiolabelled low dose: 0.8 mg/ml Radiolabelled high dose: 30 mg/ml	
3.3.6 Post-exposure period	168 hours (7 days)	
4.1 Materials and methods	**4. APPLICANT'S SUMMARY AND CONCLUSION**	
4.1.1 Treatment	(See Table A6.2.1-1) <u>Range-finding experiments</u> The high-dose level fipronil to be tested in excretion and pharmacokinetic experiments was selected on the basis of a range-finding toxicity assay with non-radiolabelled material. This assay was conducted with dose groups of 1 rat/sex involving single dose treatment of up to 150 mg/kg bw/d followed by a 8-day post-observation period. Sampling of excreta or tissues for subsequent analysis was not performed. <u>Pilot studies</u> In two pilot studies, the excretion of fipronil was investigated after single administration of 4 or 150 mg/kg bw to one male and one female rat per dose group. Excreta and expired air were recovered and sampled at regular intervals for up to 5 days post-treatment.	x
4.1.1 Treatment (cont'd)	<u>Definitive studies</u> In the main experiments groups of 5 rats/sex were either given a single nominal dose of 4 or of 150 mg/kg bw by gavage (excretion study: groups A and C, pharmacokinetic study: groups D and E). A third group of 5 rats/sex was pretreated for 14 days with non-radiolabelled fipronil at a daily dose of 4 mg/kg bw/d, before receiving a single dose of radiolabelled fipronil 24 hours later on Treatment Day 15 (excretion study: group B). In all experiments, Fipronil was administered as suspension in 0.5% aqueous methylcellulose containing also 0.01% w/v Tween 80 at a dose volume of 5 ml/kg bw. Both dose levels corresponded to a radioactive nominal dose of 25 µCi (925 kBq) per animal.	
4.1.2 Sampling	(See Table A6.2.1-2) Following dosing with [^{14}C]-fiponil, the rats were placed in all-glass metabolism cages, and samples were collected at selected time points given in Table A6.2.1-2.	

Section A6.2 **Annex Point IIA, VI.6.2**	**Absorption, distribution, metabolism and excretion of ^{14}C-Fipronil in the rat after oral administration**	
	The excreta were collected in cooled containers to prevent bacterial degradation of the metabolites present. The cages were washed with water at the times given and also with methanol at 168 hours post dose. At necropsy (168 hours after dosing) the following tissues/organs were removed for analysis; adrenals, blood, bone, brain, residual carcass, gastrointestinal (GI) tract plus contents, heart, kidneys, liver, lungs, muscle, ovaries, fat, spleen, pancreas, skin, stomach plus contents, thyroids and testes. Blood was sampled from the lateral tail vein for the pharmacokinetic groups (D and E).	
4.1.3 Radioassay	Following appropriate preparation (solubilisation or combustion) the radioactivity in the samples was measured using an LKB, Beckman or Packard Tri-Carb liquid scintillation counter. The limit of quantification was set at twice the measured background rate.	
4.1.4 Chromatography	Urine, faeces, fat, liver, kidney, muscle and uterus samples from dose groups A to C were examined for the nature and quantity of radiolabelled metabolites by HPLC. Urine samples were filtered through ACRO LC13 (0.45 µm) filters before analysis by HPLC both before and after enzymatic deconjugation with Helix pomatia containing ß-glucuonidase and arylsulfatase activity. Faecal samples were extracted successively with dichloromethane and methanol before HPLC analysis. Pooled tissue samples from fat, muscle and uterus, liver and kidney were homogenised and extracted with acetonitrile and hexane; the processing of the liver and kidney samples additionally involved extraction with methanol/water prior to HPLC analysis. Selected samples of urine, faeces or tissues (liver, kidney, muscle and fat) were each pooled and processed for identification of metabolites via GC/MS analysis.	
4.1 Results	<u>Range-finding experiments</u> At 150 mg/kg bw, the highest dose level tested, rats exhibited mild toxicity symptoms that were not considered to compromise the experiment. Therefore 150 mg/kg was chosen as the high-dose level for the definitive study. <u>Pilot studies</u> These studies indicated that pulmonary excretion was negligible (<0.25% dose) and therefore air traps were excluded from the main investigations. <u>Definitive study</u> (See Table A6.2.1-3)	**X**
Radiolabel recovery and excretion	There were no sex differences in the routes of elimination in any of the dose groups. Fipronil was relatively slowly excreted in all dose groups with means of 28.6 – 48.0% of the dose being excreted within the first 48 hours, mainly via the faeces. At sacrifice (168 hours post dose) the mean levels of radioactivity left in the tissues (including carcass) were 45.91%, 21.91% and 4.12% for Groups A, B and C respectively indicating a slow terminal elimination phase. The mean overall recoveries were ca 98% for Groups A and B and 105% for Group C.	

Section A6.2 **Annex Point IIA, VI.6.2**	**Absorption, distribution, metabolism and excretion of ^{14}C-Fipronil in the rat after oral administration**	
Absorption	(See Table A6.2.1-3) The minimum proportion of the dose absorbed may be estimated from the amounts of radioactivity found in the tissues, urine and cage washes over the seven-day study period. Using these figures the proportion of the dose absorbed appeared to be dose and dose regimen dependent with the greatest proportional absorption after the single oral low dose at 4 mg/kg (ca 53%) followed by the repeat oral low dose at 4 mg/kg (ca 39%) and the single oral high dose (ca 34%). This figure was subsequently refined using bile excretion experiments. (see report XXXX).	
Distribution	(See Table A6.2.1-4) The highest tissue residue levels at necropsy were found in the fat with moderate levels in the adrenals, pancreas, skin, liver, thyroid, ovaries and uterus. All tissues contained concentrations that were above the limits of quantification. Repeated oral dosing resulted in lower concentrations in the tissues whilst the single oral high dose produced higher concentrations albeit not proportional to the increase in dose rate (x 37.5). Concentrations in male tissues increased by a mean factor of ca 2.5 and concentrations in female tissues increased by a mean factor of ca 4.3.	
Pharmacokinetic parameters	(See Table A6.2.1-5) Fipronil and/or its radiolabelled metabolites achieved maximal concentrations in the blood moderately rapidly after a single oral dose of 4 mg/kg (ca 6 hours) and reached similar levels for both male and females (ca 0.6 – 0.7 µg equiv/g). It took significantly longer for maximal concentrations to be achieved following a single oral dose of 150 mg/kg (ca 48 – 72 hours) but the values were, again, similar between the sexes. The increase in maximal concentrations was of the order of 30 times between the two dose groups and was, therefore, practically proportional to the dose (the increase being ca 33 times between 4 and 150 and the actual mean dose rate of 131.4 mg/kg). The AUC values for the 0 – 168 hour period show the same trend (increase of ca 28 times with increase in dose). This is not reflected in the $AUC_{0\text{-}inf}$ results and is probably due to an underestimation of the terminal half-life value for the high dose level group. Using the terminal half-life values calculated from the low dose group the mean $t_{0.5}$ for fipronil was 175 hours.	**X**
Metabolites in urine	(See Table A6.2.1-6) Initial analysis of non-deconjugated urine samples indicated that the radioactivity was associated with very polar material that eluted in the void volume of the HPLC column. It was only after enzymatic deconjugation that individual components could be resolved which indicated that they were exocons derived from glucuronide conjugates. Up to 18 individual radioactive components were resolved.	**X**
Metabolites in faeces	(See Table A6.2.1-7) Up to 11 individual radioactive components were resolved in the faecal extract samples by HPLC and identified via GC-MS.	

Section A6.2 **Annex Point IIA, VI.6.2**	**Absorption, distribution, metabolism and excretion of ^{14}C-Fipronil in the rat after oral administration**	
Metabolites in tissues	Analysis of selected tissues (fat, liver, kidney, muscle and uterus) identified XXXX as being the major metabolite present which represented 100% dose in all cases except for male kidney (ca 88%) and male and female fat (ca 95%0 from the high dose groups. These results imply that XXXX was the major metabolite present 168 hours post dose. Given that ca 46% of the administered dose was present in the tissues at 168 hours post dose for the single oral low dose group and that ca 37% dose remained associated with the residual carcass it is reasonable to assume that the radioactivity ws contained within both the muscle and the fat remaining. This would yield a figure of ca 66% dose being identified for this dose group.	X
Metabolic profile	The proposed metabolic pathway, incorporating information also from the bile excretion study, is depicted at the end of section 6.2.1.	
4.3 Conclusion	Following a nominal single oral dose of fipronil to male and female rats at a dose level of 4 or 150 mg/kg bw, the rate and degree of absorption of radioactivity appeared to be dependent on dose level but not sex with slower absorption rate after administration of 150 mg/kg bw. Once absorbed fipronil was extensively metabolised and primarily released in the systemic circulation in the form of conjugates and XXXX. Conjugated fipronil metabolites were readily eliminated in the urine while XXXX was preferentially taken up by the tissues, especially in tissues with a high lipid content. The main route of elimination was the faeces, which contained, beside fipronil, up to 10 metabolites suggesting that biliary elimination might be taking place (later confirmed in the bile excretion study, see XXXX, 1995). The terminal elimination half-life of ca. 175 hours probably represents the slower release and excretion of XXXX from the tissues.	
4.3.1 Reliability	1	
4.3.2 Deficiencies	No	

Table A6.2.1-1 Dosing regime

Group	Dose	Dose level (mg/kg)	Dose route	Number of rats	Frequency of administration	Nominal Radioactive dose (µCi/rat)
Tox-1	Low	50	Oral	2	Single	0
Tox-2	Medium	100	Oral	2	Single	0
Tox-3	High	150	Oral	2	Single	0
Pilot 1	Low	4	Oral	2	Single	25
Pilot 2	High	150	Oral	2	Single	25
A	Low	4	Oral	10	Single	25
B	Low	4	Oral	10	Repeat*	25
C	High	150	Oral	10	Single	25
D	Low	4	Oral	10	Single	25
E	High	150	Oral	10	Single	25

* 14 daily doses with non-radiolabelled fipronil followed by a single oral dose of [^{14}C]-fipronil

Table A6.2.1-2 Sampling times

Group	Collection times (hours post dose)				
	Expired air	Urine	Cage wash	Faeces	Blood
Pilot 1 and Pilot 2	24, 48, 72, 96, 120	6, 24, 48, 72, 96, 120	6, 24, 48, 72, 96, 120	24, 48, 72, 96, 120	168
A, B and C	N/A	6, 24, 48, 72, 96, 120, 144, 168	6, 24, 48, 72, 96, 120, 144, 168	24, 48, 72, 96, 120, 144, 168	168
D and E	N/A	N/A	N/A	N/A	0, 0.5, 1, 2, 4, 6, 24, 48, 72, 96, 120, 144, 168

Expired air was trapped in 2-ethoxyethanol: ethanolamine (3:1 v/v)
N/A = Not applicable

Table A6.2.1-3 Recovery of radioactivity following single or repeated oral administration of [^{14}C]-fipronil

Sample	4 mg/kg single oral dose Group A						4 mg/kg repeat oral dose Group B						150 mg/kg single oral dose Group C					
	Males			Females			Males			Females			Males			Females		
	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.	Mean	±	S.D.
Urine	5.63	±	2.12	~~5.61~~ 5.62	±	1.10	16.22	±	3.38	13.80	±	~~1.33~~ 1.34	29.25	±	2.86	22.04	±	~~2.80~~ 2.81
Faeces	45.62	±	7.89	46.01	±	~~7.16~~ 7.17	56.06	±	4.43	61.36	±	~~3.35~~ 3.36	66.90	±	~~3.72~~ 3.73	75.10	±	3.44
Cage w.	0.90			~~1.19~~ 1.20			~~1.64~~ 1.65			~~3.08~~ 3.09			~~4.48~~ 4.49			~~4.00~~ 4.01		
Tissues	9.24	±	~~0.46~~ na	~~9.04~~ 9.03	±	~~0.76~~ na	5.09	±	~~0.63~~ na	~~4.98~~ 4.99	±	~~0.84~~ na	0.67	±	~~0.29~~ na	1.57	±	~~0.78~~ na
Carcass	36.81	±	8.10	36.74	±	4.12	18.57	±	3.51	15.17	±	1.89	~~2.03~~ 2.23	±	~~1.57~~ 1.40	3.76	±	~~2.68~~ 2.69
Total	98.20	±	2.32	~~98.58~~ 98.59	±	2.18	~~97.58~~ 97.59	±	0.76	~~98.40~~ 98.41	±	0.62	103.5	±	0.25	106.5	±	3.62

X
X
X
X
X

Table A6.2.1-4 Mean Concentration of radioactivity in the tissues expressed as µg equivalents of [^{14}C]-fipronil per g tissue

Tissue	4 mg/kg single oral dose Group A		4 mg/kg repeat oral dose Group B		150 mg/kg single oral dose Group C	
	Males	Females	Males	Females	Males	Females
Adrenals	4.250	4.665	1.535	1.397	7.606	14.550
Blood	0.182	0.214	0.081	0.099	1.330	2.196
Bone	0.240	0.274	0.097	0.084	0.685	1.436
Bone marrow	0.721	0.863	0.279	0.335	2.374	6.846
Brain	0.822	0.991	0.290	0.304	1.599	3.417
Fat	14.700	18.840	5.755	5.763	29.400	54.480
Carcass	1.720	1.932	0.771	0.680	3.816	6.245
GIT + C	1.373	1.699	1.142	0.892	3.676	10.490
Heart	0.992	1.187	0.361	0.408	2.294	4.529
Kidney	1.304	1.520	0.499	0.503	4.093	6.569
Liver	2.532	2.72	1.094	0.974	6.457	11.150
Lungs	1.250	1.416	0.596	0.496	3.255	5.884
Muscle	0.834	0.976	0.394	0.313	1.795	3.200
Ovaries	n/a	5.059	n/a	1.659	n/a	~~15.632~~ 15.630
Pancreas	~~3.683~~ 3.638	5.966	2.139	1.978	8.886	15.030
Skin	~~2.593~~ 2.539	3.673	1.300	1.086	7.850	17.510
Spleen	0.633	0.768	0.325	0.282	1.599	3.712
Stomach + C	0.414	0.614	0.494	0.309	0.670	2.279
Testes	0.852	n/a	0.231	n/a	1.578	n/a
Thyroid	2.266	3.483	0.876	1.524	1.448	7.705
Uterus	n/a	2.300	n/a	1.105	n/a	10.470

X

X

n/a = not applicable; GIT+C = intestinal tract + contents; Stomach+C = Stomach = contents

Table A6.2.1-5 Blood total radioactivity pharmacokinetic parameters following a single oral dose of [^{14}C]-fipronil at the levels of 4 and 150 mg/kg bw

Dose Group	Sex		Tmax (h)	Cmax (µg/g)	$t_{(0.5)}$ (h)	$AUC_{(0-168)}$ (µg equiv.h/g)	$AUC_{(0-inf)}$ (µg equiv.h/g)
Group D 4 mg/kg	Males	Mean	5.6	0.679	149.4	60.37	109.7
		SD	~~0.89~~ na	0.048	10.92	3.628	7.072
	Females	Mean	5.6	0.601	200.2	61.21	133.6
		SD	~~0.89~~ na	0.123	58.68	9.266	17.48
Group E 150 mg/kg	Males	Mean	48	19.56	54.42	1570	1716
		SD	~~0~~ na	2.903	20.1	195.3	167.9
	Females	Mean	57.6	19.72	51.22	1790	1968
		SD	~~13.15~~ na	4.735	10.5	217.6	237

Table A6.2.1-6 Mean percentage of administered dose represented by the radioactive components in the deconjugated urine

Group	Sex	Time range	% dose in sample	% dose identified				Total identified	% unidentified
				RO/1	RO/2	RPA 200766	fipronil		
A	Males	0-72	1.47	0.35	0.47	0.35	0.12	1.29	0.18
	Females	0-24	2.13	1.26	0.54	n.d.	0.03	1.83	0.30
B	Males	6-72	13.47	1.18	4.41	n.d.	0.68	6.27	7.20
	Females	0-96	14.49	1.68	3.61	0.47	1.24	7.00	7.49
C	Males	6-96	28.02	1.30	6.04	0.97	2.92	11.23	16.79
	Females	6-120	19.78	1.74	3.97	1.92	1.99	9.62	10.16

n.d. = not detected.
RO/1 & RO/2 are ring-opened metabolites; XXXX & XXXX were also indicated to be present in the report but they are known to be photolytic products and are unlikely to be rat metabolites.

Table A6.2.1.7 Mean percentage of administered dose represented by the radioactive components in the faeces

Group	Sex	Time range (h)	% dose in sample	% dose identified				Total Identified	% Unidentified [a]
				Fipronil	XXXX	XXXX	XXXX		
A	Male	0-120	30.13	13.13	0.00	1.55	11.68	26.35	3.78
	Female	0-120	25.09	10.51	0.00	1.15	9.09	20.75	4.34
B	Male	0-120	33.19	8.34	0.13	3.03	7.17	18.66	14.53
	Female	0-120	31.02	6.44	0.00	1.04	7.76	15.24	15.78
C	Male	0-120	37.14	10.61	0.78	1.30	3.83	16.51	20.63
	Female	0-120	39.99	~~18.58~~ 18.57	0.00	2.46	4.44	25.47	14.52

[a] = composed of up to 7 components.

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *XXXX* 3.1 Test material: ~~*As given in Section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in Section 2*~~ The radiolabelled test articles were repurified before use; the non-radiolabelled MB 46030 was used as delivered by the sponsor. 3.1.2.1 Description: an off-white powder 3.1.2.2 Purity: *non labelled:* ~~*99.4%*~~ >99.3% 3.1.2.3 Stability: *stable* The stability of the high dose formulation was assessed at 4 h and that of the low dose at 4 h, 4, 11 and 19 days post-formulation. 3.2.2 Strain : ~~*Charles River Crl:CD*~~ Crl :CD(SD)BR 3.2.5 Age/weight at study initiation: ~~*11 weeks; 173-304 g*~~ approximately 7-11 weeks; 167-306 g 3.3.3 Preparation of test substance: ~~*Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80*~~ Suspension in aqueous methylcellulose (0.5% (w/v)) containing Tween 80 (0.01% (w/v)).
Results and discussion	-
Conclusion	Agree with applicant's version. **Revisions/amendments:** ~~4.1~~ Results: 4.2 Results 4.1.1 Treatment: *Excreta* (urine, faeces, expired air trap solutions, cage washings and debris) *and expired air were recovered and sampled at regular intervals for up to 5 days post-treatment.* 4.2 Results : *It took significantly longer for maximal concentrations to be achieved following a single oral dose of 150 mg/kg (ca 48 – 72 hours) but the values were, again, similar between the sexes* (ca 20 µg equiv/g)*.* *Metabolites in urine: Up to* ~~*18*~~ 14 *individual radioactive components were resolved.* *Metabolites in tissues: Given that ca 46% of the administered dose was present in the tissues at 168 hours post dose for the single oral low dose group and that ca 37% dose remained associated with the residual carcass* **(**muscle and fat)
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	

Conclusion
Reliability
Acceptability
Remarks

Section A6.2 **Annex Point IIA, VI.6.2**	**Bile excretion study in the rat**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.2/03** XXXX. Fipronil: Bile Excretion Study in the rat XXXX (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 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes Safety Evaluation of Agricultural Chemicals, 59, Nohsan No 4200, January 28, 1985, Japan EU (=EEC) 94/79/EEC OECD 417	**X**
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes The protocol sacrifice time was 72 hours post-dose. 3 animals died a few hours before the time of sacrifice. Each dose suspension was prepared on the day of dosing except for the administration on the 6th April which was prepared 48 hours before. It is the opinion of the study director that these deviations did not affect the results of the study.	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	Labelled fipronil : GXR 366A Non labelled : AJK 232/C2	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	None given	X
3.1.2.2	Purity	Labelled fipronil : 99.4% Non labelled	X
3.1.2.3	Stability	Stable	
3.1.2.4	Radiolabelling	^{14}C (phenyl ring uniformly labelled)	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Charles River Crl: CD	X
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: young adult Weight : 19.76 – 341.36 g	X
3.2.6	Number of animals per group	8 (4 male and 4 female)	
3.2.7	Control animals	No	

Section A6.2 **Annex Point IIA, VI.6.2**	**Bile excretion study in the rat**	
3.2.8 Bile duct cannulation	Surgery was performed 24 hours before administration. The bile ducts of male and female rats were cannulated through a mid line incision. The cannula of each animal led through the roof of the cage to permit bile collection without disturbing the animals.	
3.3 Administration/ Exposure	Oral	
3.3.1 Type	Gavage	
3.3.2 Dose levels	40 and 4 mg/kg bodyweight	
3.3.3 Preparation of the test substance	Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80	X
3.3.4 Total volume applied	1 g dose suspension per 250 g rat bodyweight	
3.3.5 Post-exposure period	72 hours after treatment	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods		
4.1.2 Treatment	There were 2 dose levels in this study, a low dose of nominally 4 mg/kg body weight and a high dose of nominally 40 mg/kg bodyweight.	**X**
4.1.2 Sampling	Urine, faeces, cage washes (water and methanol) and bile samples were collected at 10,24, 48 and 72 hours post dose. Animals were exsanguinated whilst under anaesthesia 72 hours post dose except for 3 animals which died a few hours before due to the combined trauma of the bile duct cannulation and the administration of the test material.	**X**
	The intestinal tract, intestinal tract contents, stomach, stomach contents, cardiac blood and residual carcass were taken for radioassay.	**X**
4.1.3 Radioassay	The amounts of radioactivity in the various samples were determined by liquid scintillation counting. The limit of detection was taken to be twice the background values for blank samples in appropriate scintillation cocktails.	
4.1.4 Chromatography	The metabolites present in the bile samples were investigated using reverse phase HPLC and normal phase TLC techniques in comparison to reference standards. Enzymatic deconjugation reactions with glucuronidase and sulphatase preparations were also performed.	
4.2 Results	The absorption, distribution, metabolism and elimination of [^{14}C]-fipronil have been studied in the male and female Sprague Dawley (CD) rat following single oral administration at a nominal 40 and 4 mg/kg dose levels. The recoveries were found to be quantitative and range from 92.80% to 116.45% (mean 105.23%, SD ± 5.82%). See Table A.6.2.3-1	

Section A6.2 **Annex Point IIA, VI.6.2**	**Bile excretion study in the rat**	
4.2.1 Elimination	Both administration groups demonstrated that the elimination of radiolabel via the faeces was greater than via the bile. This, in turn, was greater than that via the urine The only exception were male rats from the High Dose Group where the levels of radioactivity were found to be highest in the bile. The highest faecal elimination was observed for rats for the High Dose Group, with 21.43% and 35.45% for the males and the females respectively. The mean levels of elimination via the faeces for the Low Dose Group reached 13.72 % and 9.74% for the males and females respectively. The mean proportion of the radioactive dose eliminated in the faeces was observed to be higher for the females than for the males for the High Dose group although this difference was not observed for the Low Dose group. The proportion of the administered dose eliminated via the biliary route was found to be significant. The mean recoveries of radioactivity in the bile for the male and female rats from the High and Low Dose Groups were: High Dose, 24.92% and 11.61%; Low Dose 7.60% and 6.76%. These results confirmed the difference between the sexes in the High Dose Group where a higher level of faecal elimination ws observed for the females. The biliary elimination observed for the males and females of the Low Dose Group was found to be similar. Low amounts of the administered dose were renally eliminated. The mean values of elimination via the urine for the male and female rats from the two Dose Groups were: High Dose, 4.66% and 2.58%; Low Dose, 0.85% and 1.62%. A greater inter-individual variability was noted for the rats from the High Dose Group. At 72 hours post dosing, the mean levels of radioactivity in the tissues (total) from the animals of the High Dose Group accounted for 55.75% (S.D. = 8.77%) for the males and 66.32% (S.D = 19.03%) for the females. The corresponding figures for the Low Dose Group were 80.17% (S.D. = 5.44%) for the males and 83.36% (S.D. 8.56%) for the females. The levels of radioactivity in the stomach contents of two of the four male rats and two of the four female rats from the High Dose Group, were found to be relatively high (14.94 % to 43.37% of the dose) at 72 hours post-dose. This result demonstrated that the absorption of Fipronil can be a relatively slow and probably passive process which may account for the observed large inter-individual variability. The intestinal tract (without contents) also displayed high levels of radioactivity, especially for rats from the Low Dose Group. This appeared to be an indication of a significant involvement of the intestine in the elimination/metabolism of fipronil and its metabolites, including a possible intestinal excretion.	**X**
4.2.2 Absorption	The proportion of the dose absorbed, estimated from the amounts of radioactivity found in urine, bile and tissues indicted that approximately 80% of the administered dose (High Dose Groups) and nearly 90% dose (Low Dose Group) had been absorbed.	**X**

Section A6.2 **Annex Point IIA, VI.6.2**	**Bile excretion study in the rat**	
4.2.3 Biliary metabolism	The metabolites of [^{14}C-fipronil analysed in the bile from males and females from both dose groups were found to be qualitatively similar. It appeared that this absorbed portion of the dose was extensively metabolised in the rat prior to secretion into the lumen of the gut. Only low amounts of unchanged compound (< 0.26% for the three day period) were detected in the bile from the High and Low Dose experiments.	**X**
	Sixteen metabolite fractions were detected in bile from the two dose groups. The main metabolite fraction observed in bile was XXXX (bile metabolite 3) which represented, for the male and the female rats respectively: High Dose Group 21.97% and 8.07%, Low Dose Group 3.136% and 1.349% over a period of 72 hours post dosing. This metabolite was more polar than the available certified standards (except XXXX). XXXX (less polar than XXXX) and XXXX) (less polar than XXXX) were the only other metabolites which represented > 0.5% of the administered dose. XXXX was found to have a retention time that corresponded to XXXX. Further metabolites, including BMET/11 (retention time corresponding to XXXX), XXXX (retention time corresponding to XXXX), were also detected in bile but accounted for < 0.5% of the administered dose.	**X**
	Following the incubation of selected bile samples with enzymatic preparations from *Helix pomatia, E. coli* and bovine liver it was found that XXXX was apparently composed of a mixture of glucuronide conjugates which liberated XXXX. The observed increases in the proportions of these metabolite fractions were dependent upon the type of ß-glucuronidase preparation that was used. XXXX also appeared to be composed of a mixture of glucuronide conjugates with XXXX representing the principal component. There were indications that XXXX could be a sulpho-conjugate although it was not possible to identify the aglycone. A significant quantitative difference was observed in the billary metabolic profiles between the High and Low Dose Experiments. The majority of radioactivity detected at 40 mg/kg being essentially represented by the conjugate XXXX which accounted for 21.7% and 8.076% in males and females. At 4 mg/kg BMET accounted for 3.136% and 1.34% for males and females respectively. See Table A.6.2.3-2.	**X**
4.3 Conclusion	Following a single oral administration, [^{14}-C]-fipronil was well absorbed at the nominal dose levels of 4 and 40 mg/kg. Total amounts recovered in urine, bile and tissues indicated that approximately 80% of the administered dose (High Dose Group) and nearly 90% (Low Dose Group) had been absorbed. The study also, showed that at least 11% (females) and 24% (males) of the radioactivity for the high dose and about 7% (both sexes) for the low dose may be eliminated in the bile in the form of conjugated (majority) and non-conjugated metabolites. The presence of XXXX, fipronil and XXXX was confirmed in the bile by the use of both HPLC and TLC techniques	
4.3.1 Reliability	1	
4.3.2 Deficiencies	None	

Table A6.2.3-1Mean percentage recoveries of radioactivity over 72 hours

X

Sample	40mg/kg		~~40~~ 4 mg/kg	
	Males	Females	Males	Females
Urine	4.66	2.58	0.85	1.62
Cage wash	1.22	1.27	0.09	0.37
Faeces	21.43	26.88	13.72	9.74
Bile	24.92	11.60	7.60	6.76
Tissues	55.75	66.32	80.17	83.36
Total	**107.98**	**108.65**	**102.43**	**101.85**

Table A6.2.3-2 Mean percentage of administered dose represented by radioactive components in the bile over 72 hours

X

BMET/	Percentage of administered dose				Chromatographic identification[a]
	40mg/kg		~~40~~ 4 mg/kg		
	Males	Females	Males	Females	
1	0.030	n.d.	0.095	n.d.	-
2	0.544	n.d.	0.163	0.010	-
3	21.971	8.076	3.136	1.349	Conjugates[b]
4	0.028	0.244	n.d.	n.d.	-
5	0.726	1.697	0.897	2.976	Conjugates[c]
6	0.307	0.329	0.009	n.d.	-
7	0.774	0.569	1.368	0.688	XXXX
8	0.051	0.067	0.098	0.201	-
9	0.055	0.103	0.124	0.258	-
10	0.088	0.049	0.220	0.105	-
11	0.086	0.104	0.243	0.334	XXXX
12	0.087	0.065	0.268	0.227	-
13	0.120	n.d.	0.249	0.135	-
14	0.005	n.d.	0.024	n.d.	-
15	0.090	0.148	0.253	0.264	Fipronil
16	0.048	0.154	0.466	~~0.205~~ 0.206	XXXX

X

n.d. not detected

[a] Components possessed similar chromatographic properties to certified standards using HPLC and TLC techniques

[b] Incubation with enzymatic preparations from *E Coli, Helix pomatia* and bovine liver indicated that this radiolabelled component was essentially a mixture of at least 5 glucuronide conjugates

[c] Incubation with enzymatic preparations from *E Coli, Helix pomatia* and bovine liver indicated that this radiolabelled component was essentially a mixture of at least 6 glucuronide conjugates.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *XXXX* 2.1 Guideline study: ~~*EU (=EEC) 94/79/EEC*~~ 87/18/EEC 3.1 Test material: ~~*As given in Section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in Section 2*~~ The radiolabelled test articles were repurified before use; the non-radiolabelled MB 46030 was used as delivered by the sponsor. 3.1.2.1 Description: ~~*None given*~~ a white powder 3.1.2.2 Purity: labelled fipronil: ~~*99.4%*~~ 98.8% ; *non labelled:* 99.4% 3.1.2.3 Stability: *stable* 3.2.2 Strain : ~~*Charles River Crl:CD*~~ Crl :CD(SD)BR 3.2.5 Age/weight at study initiation: ~~*19.76-341.36g*~~ 219.76-341.36 g 3.3.3 Preparation of test substance: ~~*Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80*~~ Suspension in aqueous methylcellulose (0.5% (w/v)) containing Tween 80 (0.01% (w/v)).
Results and discussion	-
Conclusion	Agree with applicant's version. **Revisions/amendments:** ~~*4.1.2 Treatment:*~~ 4.1.1 Treatment 4.1.2 Sampling: *Urine, faeces, cage washes (water and methanol) and bile samples were collected at* the following intervals : urine and faeces: 0, 0-*10*,10-*24*, 24-*48 and* 48-*72 hours post dose.* Bile: 0, 0-5, 5-10, 10-24, 24-48, 48-72 hours after dosing. *The intestinal tract, intestinal tract contents, stomach, stomach contents, cardiac blood and* skin and fur were removed from each animal after exsanguination. The *residual carcass* ~~*were*~~ was *taken for radioassay.* Metabowl Cage washes with HPLC-grade water and methanol were taken at the intervals 0-10, 10-24, 24-48 and 48-72 hours post dosing. ~~*4.2.2 Absorption*~~ 4.2.2 Distribution: *At 72 hours post dosing, the mean levels of radioactivity in the tissues (total) from the animals of the High Dose Group accounted for 55.75% (S.D. = 8.77%) for the males and 66.32% (S.D = 19.03%) for the females. The corresponding figures for the Low Dose Group were 80.17% (S.D. = 5.44%) for the males and 83.36% (S.D. 8.56%) for the females.* ~~*4.2.2 Absorption*~~ 4.2.3 Absorption ~~*4.2.3 Biliary metabolism*~~ 4.2.4 Biliary metabolism

	4.2.3 Biliary metabolism: ~~*XXXX (retention time corresponding to XXXX)*~~ XXXX (retention time corresponding to Fipronil) and XXXX (retention time corresponding to XXXX) *XXXX which accounted for ~~21.7%~~* 21.971% *and 8.076% in males and females* from the High Dose Group.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Materials and methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vivo* dermal absorption study in rats with Fipronil WG formulation**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.2/04** XXXX Dermal absorption of ^{14}C-Fipronil Regent 80 WDG in male rats (Preliminary and definitive phases). 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 1.	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: USEPA 85-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Regent 80 WDG (BASF code: BAS 350 00 I; water-dispersible granule formulation containing nominal 80% Fipronil) spiked with ^{14}C-radiolabelled Fipronil Please see IIIA 6.2 - BCI	
3.1.1	Lot/Batch number	Radiolabelled fipronil: batch no. GHS-826 Non labelled Regent 80 WDG: batch no. OP930794	
3.1.2	Specification	Not applicable	X
3.1.2.1	Description	Off-white solid powder	
3.1.2.2	Purity	Radiolabelled Fipronil: 98% Fipronil content in Formulation: 789 g/kg	
3.1.2.3	Stability	stable	X
3.1.2.4	Radiolabelling	^{14}C- label uniform at phenyl moiety	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Charles River Crl:CD	X
3.2.3	Source	XXXX	
3.2.4	Sex	Male	
3.2.5	Age/weight at study initiation	8 Weeks 192 – 214 g	X
3.2.6	Number of animals per group	24	
3.2.7	Control animals	Yes : 2 per group	X
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test substance	Suspension in 1% carboxymethyl cellulose solution	X
3.3.2	Concentration of test substance	0.07 mg/cm², 0.668 mg/cm², 3.88 mg/cm², corresponding to nominal fipronil concentrations in dose suspensions of 0.9%, 8% and 39%	X
3.3.3	Specific activity of test substance	19.8 mCi/mmol	
3.3.4	Volume applied	100 µl	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vivo* dermal absorption study in rats with Fipronil WG formulation**	

3.3.5 Size of test site 3.3.6 Exposure period 3.3.7 Sampling time 3.3.8 Samples	12.5 cm^2 0.5, 1, 2, 4, 10 and 24 h 0.5, 1, 2, 4, 10 and 24 h after initiation Urine, faeces, carcass, skin with substance not removable, liquid used for washing the skin	
	4. RESULTS AND DISCUSSION	
4.1 Toxic effects, clinical signs	No clinical signs were observed	
4.2 Dermal irritation	None recorded	
4.3 Recovery of labelled compound	99.8 – 116%	**x**
4.4 Percutaneous absorption	(See also Table A6.2.4-1)	

	Direct absorption		Treatment site skin	
	10 h	24 h	10 h	24 h
Low dose 0.876 mg/animal	0.65%	0.36%	1.87%	1.82%
Mid dose 8.35 mg/animal	0.02%	0.40%	1.57%	3.29%
High dose 48.5 mg/animal	0.18%	0.07%	0.69%	0.49%

Between 96–103% of the administered dose was recovered in the skin wash following 10-24 hour exposure, indicating that fipronil could be almost quanititatively washed off even after prolonged exposure. Directly absorbed radiolabel (based on recoveries in blood, excreta and carcass) was very low after 10- or 24-h exposure, accounting for less than 1% of the administered dose. Between 1-3% of the administered dose was not washed off from the treatment site skin, but the kinetic data did not suggest that a substantial proportion of this skin residue would become systemically bioavailable	**x**
5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	The commercial fipronil product BAS 350 00 I (trade name Regent 80 WDG), a water-dispersible granular formulation, was spiked with radiolabelled (^{14}C) Fipronil and administered topically to groups of male rats in order to assess the dermal absorption of radiolabelled Fipronil from this formulation. Dose suspensions were prepared by mixing (unlabelled) BAS 350 00 I with known amounts of ^{14}C-radiolabelled Fipronil using 1% CMC to provide the required nominal dose levels. Dose levels were selected based on the results of a preliminary study in which 4 male rats were treated with nominal dose levels of either 0.8 or 40 mg of Fipronil/animal. Groups of 24 male Sprague-Dawley CD rats were given a single topical application of 100 µl of either 0.8, 8 or 40 mg of ^{14}C-Fipronil-labelled BAS 350 00 I suspended in 1% carboxymethylcellulose (CMC) as the carrier. A group of 2 males received the carrier alone as controls. The dose was applied to a shaved area on the back/shoulders of each animal protected by a plastic enclosure (ca 12.5 cm^2) that was glued to the skin surface. The treated site was covered with non-occlusive filter paper and each animal wore an Elizabethan collar to protect the application site.

Section A6.2 **Annex Point IIA, VI.6.2**	***In vivo* dermal absorption study in rats with Fipronil WG formulation**	
	Animals were observed twice daily for mortality and once daily for clinical signs (general health and appearance). Individual bodyweight was recorded at arrival, at randomisation and on the day prior to dosing. Samples of urine and faeces were collected from each rat. Groups of 4 males were killed 0.5, 1, 2, 4, 10 and 24 hours after dosing. Control animals were sampled 24 hours post-dose. Just prior to necropsy, the animals were anaesthetised and the skin washed with gauze pads and cotton applicators moistened with soap and water. The pads and applicators were retained for subsequent analysis for radioactivity. At necropsy, a blood sample and residual urine from the bladder was collected for quantification of radioactivity. The non-occlusive filter paper cover, application site enclosure, skin wash, cage wash and wipes, skin from the treated site and the carcass, were also quantified. Radioactivity was quantified by liquid scintillation counting. Samples of faeces, all blood and the residual carcass, were combusted prior to quantification. Aliquots of the dose preparations were analysed pre- and post-dosing to confirm radioactivity levels, homogeneity as well as radiochemical and chemical purities. The rats were about 7 weeks old and weighed 192 to 214 g (preliminary phase) and 158 to 217 g (main study) at receipt. They were housed individually and were acclimatised for at least 7 days. During the main study they were held in metabolism cages for the separation of faeces and urine.	X
5.2 Results and discussion	<u>Analysis of dose preparations</u> HPLC analysis confirmed the radiochemical purity of ^{14}C-Fipronil as 98.06% and that suspensions in 1% carboxymethylcellulose were stable following refrigerated storage for 2 days. Furthermore, it confirmed that the dose levels administered were close to the target values. Homogeneity of mixing was also verified. Mortality and clinical signs There were no deaths and no clinical signs of toxicity. Mass balance In the preliminary study, mean recoveries at the nominal doses levels of 0.8 and 40 mg/rat were 102% and 116% respectively, with the majority of radioactivity found in the skin wash (100 and 116% respectively). Small amounts were retained in or on the skin application site (0.39 and 0.23%, respectively). In the main study, radioactivity in samples from the control group was below the limit of detection. Overall mean recoveries for the treated groups for all time points were 99.8, 101 and 104% of the total at nominal dose levels of 0.8, 8 and 40 mg/rat, respectively. The majority of radioactivity was not systematically absorbed and was washed off the skin application site. These recoveries accounted for 96.2 to 98.8%, 95.4 to 101% and 101 to 105% of the total radioactivity at 0.8, 8 and 40 mg/rat, respectively. Generally, the amount of activity detected in or on the treated skin site accounted for <3.3% of the total dose, except at 1 hour after 8 mg/rat where an outlier value of 5.75% was obtained. The amount of radioactivity in the excreta, including the cage wipe and wash, was <0.02% of the total applied.	X

Section A6.2 **Annex Point IIA, VI.6.2**	***In vivo* dermal absorption study in rats with Fipronil WG formulation**	
	Absorption (See Table A6.2.4-1) Quantities of radioactivity recovered in the blood, eliminated in the excreta and retained in the carcass were considered to result from direct dermal absorption of Fipronil. The quantity of radiolabel recovered at the application site skin despite of washing could be considered to be potentially absorbable. At 0.5 hours post-dosing, the amount of direct and indirect absorption was not detectable at any dose level. At 24 hours, direct/potential absorption was 0.36/2.19%, 0.40/3.69% and 0.07/0.55% a nominal dose levels of 0.8, 8 and 40 mg/rat, respectively. In terms of mg of Fipronil equivalents, the amount of direct/indirect absorption was linear at 0.8 and 8 mg/rat (0.003/0.019 and 0.033/0.308 mg, respectively). Corresponding values at 40 mg/rat were 0.034/0.267 mg. This highest dose level was approximately 100-fold higher and about 10-fold higher than the lowest and intermediate dose levels, respectively. These results at 24 hours suggested a linear dose-absorption relationship at the lower dose levels but that saturation had been reached at the highest dose level. Overall, absorption estimates were very low, mean values obtained at the exposure/sacrifice time points of between 2 and 24 hours ranging between 0.005–0.65% (low dose), 0.02–0.40% (mid dose) and 0.07–0.18% (high dose). There was no obvious time-dependent increase in the absorption estimates (neither a corresponding decrease of radiolabel in the application site skin), suggesting that the residues at the application site skin were not appreciably bioavailable during the 24-h observation period.	**X** **X**
5.3 Conclusion	Dermal absorption of Fipronil in male rats following topical application of BAS 350 00 I (Regent 800 WDG) spiked with ^{14}C-radiolabelled Fipronil was very low. Results obtained 24 hours post-dosing indicated that absorption was linear at the lower dose levels (nominally 0.8 and 8 mg of Fipronil/rat) but that saturation had been reached at the highest (nominal) dose level, 40 mg/rat. The majority of the radioactivity was not absorbed but was removed during the skin wash. Less than 0.7% of the Fipronil dose was dermally absorbed during exposure periods of up to 24 hours irrespective of the dose level tested. Between 1-3% of the dose was still associated with the application site skin after washing, but the kinetic data did not allow to conclude that a substantial fraction thereof would be available for subsequent absorption (i.e. no obvious time-dependent increase of absorption). Additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations and a higher penetration rate through rat compared to human skin.	**X**
5.3.1 Reliability	2	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vivo* dermal absorption study in rats with Fipronil WG formulation**	
5.3.2 Deficiencies	The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. This is not considered to lead to underestimation of fipronil's dermal absorption potential, since the presence of formulants usually lead to enhanced absorption. Furthermore, additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations.	

Table A6.2.4-1 Table for Percutaneous Absorption (in vivo test)

Sampling time (hours)	Amount of radioactivity recovered (% of dose)												
	Cover	Enclosure	Skin wash	Treated skin site	Blood	Carcass	Cage wash	Cage wipe	Urine	Faeces	Total recovery	Absorbed [a]	Absorbed + recovery in treatment-site skin
0.8 mg/animal													
0.5	ND	0.30	98.8	1.14	ND	ND	ND	ND	<0.005	ND	100	<0.005	1.14
1	ND	0.22	98.7	1.51	ND	0.07	ND	ND	ND	ND	100	0.07	1.58
2	ND	0.17	97.9	2.45	ND	0.46	ND	ND	ND	ND	101	0.46	2.91
4	ND	0.11	97.8	1.86	ND	ND	ND	ND	<0.005	ND	99.7	<0.005	1.86
10	ND	0.29	96.2	1.87	ND	0.65	ND	ND	<0.005	ND	99.0	0.65	2.52
24	ND	0.09	96.8	1.82	ND	0.36	ND	ND	0.01	ND	99.1	0.36	2.19
8 mg/animal													
0.5	0.01	0.19	101	0.60	ND	ND	ND	ND	ND	ND	101	ND	0.61
1	0.09	0.27	95.4	5.75	ND	0.06	ND	ND	ND	ND	101	0.06	5.82[1]
2	<0.005	0.21	101	0.85	ND	0.05	ND	ND	<0.005	ND	102	0.05	0.90
4	0.01	0.09	100	1.58	ND	ND	ND	ND	ND	0.10	101	0.10	1.65
10	0.01	0.19	101	1.57	ND	ND	ND	0.01	<0.005	0.01	103	0.02	1.59
24	0.01	0.18	97.1	3.29	ND	0.38	ND	ND	0.01	0.01	100	0.40	3.69
40 mg/animal													
0.5	0.01	0.06	105	0.35	ND	ND	ND	ND	ND	ND	105	ND	0.35
1	ND	0.15	101	0.8	ND	0.64	ND	ND	ND	ND	103	0.64	1.44
2	0.01	0.07	103	0.35	ND	0.05	ND	ND	ND	ND	104	0.05	0.40
4	0.01	0.11	101	0.76	ND	0.07	ND	ND	ND	ND	102	0.07	0.83
10	0.01	0.16	103	0.69	ND	0.18	ND	ND	<0.005	<0.005	104	0.18	0.87
24	0.01	0.11	103	0.49	ND	0.07	ND	ND	<0.005	ND	104	0.07	0.55

[a] Total radioactivity from blood, carcass, cage wash and wipe, urine and faeces

[1] due to an outlier value of 17.2% (0.99, 4.17, 0.91, 17.2); mean value for indirect absorption without this value would be 2.02%

ND Not detectabl

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1.2 Specification: ~~*Not applicable*~~ The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. 3.1.2.3 Stability: The stability of the dosing suspensions for the duration of the test period was determined by Hazleton Wisconsin Inc. 3.2.2 Strain : *Charles River Crl:CD* ®BR 3.2.5 Age/weight at study initiation: ~~*192- 214 g*~~ 158-217 g 3.2.7 Control animals: *Yes : 2 per group* treated with the vehicle only (1% carboxymethylcellulose). 3.3.1 Preparation of test substance: Dose suspensions were prepared by combining known amounts of ^{14}C-fipronil, nonlabeled Regent 80 WDG, and ~~*Suspension in*~~ *1% carboxymethyl cellulose solution.* 3.3.2 Concentration: *0.07 mg/cm², 0.668 mg/cm², 3.88 mg/cm², corresponding to nominal fipronil concentrations in dose suspensions of 0.9%, 8% and 39%,* corresponding to 0.876, 8.35 and 48.5 mg/animal.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.3 Recovery of labelled compound: ~~*99.8%-116%*~~ The overall mean recoveries, including all time points, were 99.8%, 100% and 104% of the total dose for the 3 tested groups. 4.4 Percutaneous absorption : ~~*Between 1-3% of the administered dose was not washed off from the treatment site skin,*~~ Among the tested groups, skin of the test site generally accounted for less than 3.3% (an outlier of 5.75% was observed at 1 hour postdose for the group treated with 8.35 mg/rat) of the total applied radioactivity.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *The non-occlusive filter paper cover, application site enclosure, skin wash, cage wash and wipes, skin from the treated site,* feces *and the carcass, were also quantified.* 5.2 Results and discussion: *Overall mean recoveries for the treated groups for all time points were* ~~*99.8, 101*~~ 99.1, 100 *and 104% of the total* *The quantity of radiolabel recovered at the application site skin despite of washing could be considered to be potentially absorbable.* The sum of direct absorption and amounts left on/in the skin of the test site after skin wash was considered as the amount of indirect absorption of fipronil.

	~~*and 0.07- 0.18% (high dose)*~~ and 0.05-0.18% (high dose) 5.3 Conclusion: ~~*Between 1-3% of the dose was still associated with the application site skin after washing*~~ Among the tested groups, skin of the test site generally accounted for less than 3.3% (an outlier of 5.75% was observed at 1 hour postdose for the group treated with 8.35 mg/rat) of the total applied
Reliability **Acceptability** **Remarks**	2 –The study was not made with the pure substance but with a formulation. acceptable
	COMMENTS FROM …
Date **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil ULV formulation**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.2/05** XXXX. Fipronil: In vitro absorption from a 25 g/l ULV formulation through human and rat epidermis 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 1.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Not available at the time the study was conducted; in general compliant to OECD 428 (adopted 2004)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	BAS 350 50 I (syn. ADONIS, Commercial fipronil ULV formulation containing nominal 25 g/l Fipronil) and an oil-based 1:10 v/v spray strength dilution thereof Please see IIIA 6.2 - BCI	
3.1.1 Lot/Batch number	Formulation concentrate: EXP 619 13A, batch no. 1880 FD 96 Formulation dilution: CRLD 967096, batch no. 1897 FD 96	**X**
3.1.2 Specification	Concentrate: 26 g Fipronil/L (analysed); Dilution: 2.5 g Fipronil/L (nominal)	
3.1.2.1 Description	Bright-red liquid	
3.1.2.2 Purity	Fipronil content in Formulation: 26 or 2.5 g/l	**X**
3.1.2.3 Stability	stable	**X**
3.1.2.4 Radiolabelling	^{14}C- label uniform at phenyl moiety	
3.2 Test system		
3.2.1 Rat epidermis	Epidermis were prepared from male Wistar rats aged ca.28 days. Subcutaneous fat was removed, Skins wer soaked for ca. 20 hours in 1.5 M Sodium bromide, washed and the epidermis carefully peeled from the dermis	
3.2.2 Human epidermis	Underlying muscle and fat were removed from human whole skin samples. The skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis separated from the dermis.	
3.2.4 Chamber	Horizontal glass diffusion cells, static conditions, receptor chamber maintained at 30 ± 1°C	
3.2.5 Membrane integrity check	Yes The integrity of the epidermal membranes was determined by measurement of their electrical resistance. Membranes with a measured resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used .	
3.2.6 Number of cells per group	6/concentration/species	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil ULV formulation**
3.2.7 Reference substances	No
3.2.8 Receptor fluid	50% v/v ethanol in distilled water
3.2.9 Quantification of radioactivity	Liquid Scintillation Counting
3.2.9 Endpoints	Skin permeation (flux) , % applied dose penetrated, mass balance
3.3 Administration/ Exposure	
3.3.1 Preparation of test substance	Samples were tested as supplied
3.3.2 Concentration of test substance	Concentrate: 26 g/l Dilution: 2.5 g/l
3.3.3 Specific activity of test substance	Concentrate: 4.75 MBq / 2.75 g Dilution: 7.1 MBq / 3.50 g
3.3.4 Volume applied	10 µl/cm²
3.3.5 Area of skin	2.54 cm²
3.3.6 Exposure period	24 h
3.3.7 Sampling time	Time-Absorption diagrams indicate that samples were obtained at the following time points post application: 1, 2, 3, 4, 6, 8, 10, 12, 18, 20, 22, and 24 h.
	4. RESULTS AND DISCUSSION
4.1 Penetration rate Flux (µg/cm²/h)	See Table A6.2.5-1 Within 24 hours of exposure, the following mean fluxes could be calculated through rat / human epidermis: Undiluted formulation: 0.449 / 0.037 µg/cm²/h 1:10 diluted formulation: 0.110 / 0.009 µg/cm²/h
4.2 % radiolabel permeated	(See Table A6.2.5-1) Within 6–10 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 1.2–2.1% / 0.15–0.18 % 1:10 diluted formulation: 2.1–3.9% / 0.17–0.29% Within 24 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 4.3 / 0.32 % 1:10 diluted formulation: 10.3 / 0.86 %

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil ULV formulation**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	^{14}C-radiolabelled and unlabelled Fipronil was formulated in a ultra-low-volume liquid (ULV) formulation to produce a nominal 25 g Fipronil/l concentrate (corresponding to the commercial formulation BAS 350 50 I) and an oil-based 1:10 v/v dilution thereof (nominal 2.5 g Fipronil/l). Using these formulations, the *in vitro* penetration of Fipronil through human and rat epidermiswas assessed. Human and rat epidermal skin, excluding the dermis and underlying fat/muscle, with an electrical resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used. Samples of each epidermis were exposed to the concentrate and spray dilution as follows. Six glass diffusion cells were used for each concentration and test species. A sample of epidermal skin was mounted on a support grid in the chamber of a glass diffusion cell. 10 µl/cm² of the concentrate or dilution was applied to approx. 2.54 cm^2 of the skin preparation. A recorded volume of receptor fluid (50% v/v ethanol in distilled water) was put in the receptor beneath the mounted skin sample and the cell placed in a water bath maintained at 30 $\pm$ 1°C. The epidermis was unoccluded for the 24-hour exposure period to simulate potential human dermal exposure during normal use. At recorded intervals pre- and post-dosing, samples (0.1 ml) of the chamber fluid were taken and replaced by fresh fluid. The radioactivity in each sample was quantified by liquid scintillation to determine the amount of Fipronil. At the end of the exposure period, each epidermis sample was carefully decontaminated by washing and digested. Radioactivity in the washings and the digested epidermis were quantified and the mass-balance of the various fractions and the distribution of radiolabel were determined.	**X**
5.2 Results and discussion	Absorption (see Table A6.2.5-1) The rate of absorption of Fipronil through the human epidermis from both the concentrate and oil-based spray dilution was essentially unchanged throughout the entire 24-hour exposure period (means 0.037 and 0.009 µg/cm^2/h, respectively). Although absorption rates through the rat epidermis were also essentially unchanged over this same period (0.449 and 0.110 µg/cm^2/h for the concentrate and dilution, respectively), penetration was about 12 times faster than through the human epidermis.	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil ULV formulation**	
	During a typical 6-10 hour working day, the mean amount of Fipronil absorbed from the concentrate through human epidermis was 0.39-0.47 µg/cm^2 and 3.2-5.4 µg/cm^2 through the rat. These values corresponded to 0.15-0.18% and 1.2-2.1% of applied dose for human and rat specimens, respectively. For the spray dilution, whilst the quantity absorbed was lower (0.042-0.072 and 0.54-0.97 µg/cm^2, respectively), the percentage of the applied dose was higher (0.17-0.29% and 2.1-3.9% in human and rat epidermis, respectively). After 24-hours of exposure to the concentrate, 0.83 and 11.2 µg/cm^2 of Fipronil (0.32 and 4.3% respectively) had been absorbed by human and rat epidermis, respectively, whilst 0.21 and 2.6 µg/cm^2 of the dilution were absorbed by these two matrices, respectively (0.86 and 10.3% of the respective applied dose). Therefore, after 24 hours, a higher percentage of the applied dilution dose was absorbed by both human and rat epidermis than of the concentrate. <u>Mass balance and distribution</u> (see Table A6.2.5-2) Only the quantity of Fipronil in the receptor fluid was considered to be absorbed. Amounts from the spreader, donor chamber, rinsings and the epidermis itself were considered unavailable for absorption. Fipronil in the epidermis was also deemed unabsorbed since it will most likely be lost *in vivo* by desquamation. In the human epidermis the majority of the applied dose (88 and 65 % for concentrate and dilution, respectively) was washed off during decontamination. Similarly, in the rat, 79 and 60% of the applied doses of the concentrate and dilution, respectively, was released by decontamination. For human epidermis, about 25 and 40% of the concentrate and dilution, respectively, were deemed unavailable whilst the corresponding values for rat epidermis were slightly lower (15 and 30%, respectively). Following application of the concentrate and dilution, the amounts of Fipronil in the human epidermis (4.14% and 2.64%, respectively) were markedly lower than corresponding values for the rat epidermis (10 and 25% respectively). The amount of Fipronil absorbed through the human epidermis during the 24-hour exposure period was 0.3% of the applied dose of the concentrate and 0.86% for the dilution. In the rat, 4.3 and 10% of the applied dose of the concentrate and dilution, respectively, was absorbed.	**x**
5.3 Conclusion	Absorption of Fipronil through the human epidermis was slow for both the concentrate and the oil-based spray dilution of a 25 g/l ULV formulation (BAS 350 50 I). The data predicted that dermal absorption from both the concentrate and spray dilution would be low during typical exposure periods (6-10 hours) and that the majority of any Fipronil in contact with human skin would be removed during normal washing procedures. Furthermore, as a model for predicting dermal absorption of this compound, the rat overestimates absorption of Fipronil through human skin. Flux rates using rat epidermis were about 12-fold higher than using human epidermis.	
5.3.1 Reliability	2	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil ULV formulation**

5.3.2 Deficiencies	The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. This is not considered to lead to underestimation of fipronil's dermal absorption potential, since the presence of formulants usually lead to enhanced absorption. Furthermore, additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations.	

Table A6.2.5-1 Summary of absorption of Fipronil through human and rat epidermis following application of the concentrate and spray dilution of a 25 g/l ULV formulation

Time (hours)	**Concentrate (25 g/l)**		**Dilution (2.5 g/l)**	
	Rat	**Human**	**Rat**	**Human**
Mean absorption rates (µg/cm^2/h)				
1-24	0.449	0.037	0.110	0.009
Mean amount absorbed (µg/cm^2)				
6	3.2	0.39	0.54	0.042
8	4.4	0.42	0.69	0.054
10	5.4	0.47	0.97	0.072
24	11.2	0.83	2.6	0.21
% of dose absorbed				
6	1.2	0.15	2.1	0.17
8	1.7	0.16	2.8	0.22
10	2.1	0.18	3.9	0.29
24	4.3	0.32	10.3	0.86

Table A6.2.5-2 Mass balance and distribution of Fipronil following application of the concentrate and spray dilution of a 25 g/l ULV formulation (BAS 350 50 I) to human and rat epidermis (mean % of dose recovered)

	Human		**Rat**	
	Concentrate (25 g/l)	**Dilution** (2.5 g/l)	**Concentrate** (25 g/l)	**Dilution** (2.5 g/l)
Spreader	7.68	17.2	3.86	4.19
Donor chamber	18.5	24.1	12.0	26.1
Skin wash	87.6	65.4	78.6	60.4
Epidermis	4.14	2.64	10.1	24.7
Absorbed	0.30	0.86	4.29	10.3
Total	118	110	109	126

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1.1 Lot/Batch number: ~~*EXP 619 13A*~~ EXP60913A 3.1.2.2 Purity/3.1.2.3 Stability: The purity and stability of the spray dilution are the responsibility of the sponsor.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *to produce a nominal 25 g Fipronil/l concentrate* (actual content 26g Fipronil/l) 5.2 Results and discussion Mass balance and distribution: ~~*Only the quantity of Fipronil in the receptor fluid was considered to be absorbed. Amounts from the spreader, donor chamber, rinsings and the epidermis itself were considered unavailable for absorption. Fipronil in the epidermis was also deemed unabsorbed since it will most likely be lost in vivo by desquamation.*~~ The quantity of fipronil in the receptor fluid was considered to be absorbed directly and the quantity in the epidermis was considered to be available to be absorbed, indirectly. In the OECD 428 guideline, it is written that "the test substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid values alone".
Reliability **Acceptability** **Remarks**	2 –The study was not made with the pure substance but with a formulation. acceptable
	COMMENTS FROM …
Date **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil EC formulation**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.2/06** XXXX. Fipronil: In vitro absorption from 300 g/l EC formulation through human and rat epidermis 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 1.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Not available at the time the study was conducted; in general compliant to OECD 428 (adopted 2004)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	a) BAS 350 43 I (= EXP 61196A = Regent 2.5 EC): Commercial EC formulation with nominal Fipronil content: 300 g/l containing ^{14}C-radiolabelled Fipronil Please see IIIA 6.2 - BCI b) CRLD 967095: 1:50 (v/v) aqueous dilution of EC formulation BAS 350 43 I, 6 g/l Fipronil containing ^{14}C-radiolabelled Fipronil	
3.1.1 Lot/Batch number	a) 1878 FD 96; Radiolabelled fipronil ref. no. 4950 b) 1896 FD 96; Radiolabelled fipronil ref. no. 4954	
3.1.2 Specification	a) 1878 FD 96: 312 g Fipronil/L; b) 1896 FD 96: 6 g Fipronil/L	
3.1.2.1 Description	a) Luminous yellow liquid b) White liquid	
3.1.2.2 Purity	Fipronil content in Formulation: 312 g/l or 6 g/l	**X**
3.1.2.3 Stability	stable	**X**
3.1.2.4 Radiolabelling	^{14}C- label uniform at phenyl moiety	
3.2 Test system		
3.2.1 Rat epidermis	Epidermis were prepared from male Wistar rats aged ca.28 days. Subcutaneous fat was removed, Skins wer soaked for ca. 20 hours in 1.5 M sodium bromide, washed and the epidermis carefully peeled from the dermis	
3.2.2 Human epidermis	Underlying muscle and fat were removed from human whole skin samples. The skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis separated from the dermis.	
3.2.4 Chamber	Horizontal glass diffusion cells, static conditions, receptor chamber maintained at 30 ± 1°C	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil EC formulation**	
3.2.5 Membrane integrity check	Yes The integrity of the epidermal membranes was determined by measurement of their electrical resistance. Membranes with a measured resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used.	
3.2.6 Number of cells per group	Concentrate: 6/species Dilution: 5/species	**x**
3.2.7 Reference substances	No	
3.2.8 Receptor fluid	50% v/v ethanol in distilled water	
3.2.9 Quantification of radioactivity	Liquid Scintillation Counting	
3.2.9 Endpoints	Skin permeation (flux), % applied dose penetrated, mass balance	
3.3 Administration/ Exposure		
3.3.1 Preparation of test substance	The samples were tested as supplied.	
3.3.2 Concentration of test substance	Concentrate: 312 g/l Dilution: 6 g/l	
3.3.3 Specific activity of test substance	Concentrate: 4.4 MBq / 3.43 g Dilution: 6.8 MBq / 3.45 g	
3.3.4 Volume applied	10 µl/cm²	
3.3.5 Area of skin	2.54 cm²	
3.3.6 Exposure period	24 h	
3.3.7 Sampling time	Not specifically mentioned in the report. Time-sbsorption diagrams indicate that samples were obtained at the following time points post application: 1, 2, 3, 4, 6, 8, 10, 12, 18, 20, 22, 24 h.	
4.1 Penetration rate Flux (µg/cm²/h)	**4. RESULTS AND DISCUSSION** (See Table A6.2.6-1) Within 24 hours of exposure, the following mean fluxes could be calculated through rat / human epidermis: Undiluted formulation: 1.03 / 0.215[a] µg/cm²/h 1:50 aqueous dilution: 0.185 / 0.041 µg/cm²/h [a] Fipronil absorption of the concentrate was only noted in 1/5 cells tested; the LOQ value of 0.186 µg/cm²/h was substituted for the other 4 cells to allow calculation of the mean.	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil EC formulation**	

4.2	**% radiolabel permeated**	(See Table A6.2.6-1) Within 6–10 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 0.34–0.43% / 0.14–0.15 % 1:50 diluted formulation: 6.4–7.1% / 0.22–0.49% Within 24 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 0.82 / 0.15 % 1:50 diluted formulation: 10.3 / 1.5 %	
5.1	**Materials and methods**	**5. APPLICANT'S SUMMARY AND CONCLUSION** Radiolabelled [^{14}C] samples of the concentrated 300 g/l (nominal) of fipronil EC formulation BAS 350 43 I and a 1:50 v/v aqueous spray strength dilution containing nominal 6 g/l of fipronil, were used to assess the absorption of fipronil *in vitro* through human and rat epidermis. Human and rat epidermal skin, excluding the dermis and underlying fat/muscle, with an electrical resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used. Samples of each epidermis were exposed to the concentrate and spray dilution as follows. A sample of epidermis was placed on a support grid in the chamber of a glass diffusion cell. 10 µl/cm² of the concentrate or dilution was applied to approx. 2.54 cm^2 of the skin preparation. A recorded volume of receptor fluid (50% v/v ethanol in distilled water) was put in the receptor beneath the mounted skin sample and the cell placed in a water bath maintained at 30 ± 1 °C. The epidermis remained unoccluded for the 24-hour exposure period to simulate potential human dermal exposure during normal use. At recorded intervals pre- and post-dosing, samples (0.1 ml) of the receptor chamber fluid were taken and replaced by fresh fluid. The radioactivity in each sample was quantified by liquid scintillation to determine the amount of Fipronil. At the end of the exposure period, each epidermis sample was carefully decontaminated by washing and digested. Radioactivity in the washings and the digested epidermis were quantified and the mass-balance of the various fractions and the distribution of radiolabel were determined.	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil EC formulation**	
5.2 Results and discussion	Absorption (see Table A6.2.6-1) Absorption of Fipronil through the human epidermis from the concentrate was detected in only 1 of the 5 cells (0.329 µg/cm^2/h). Therefore, the LOQ (0.186 µg/cm^2/h) was used for the other 4 cells in order to calculate the mean absorption rate (0.215 µg/cm^2/h). For the aqueous spray dilution, the absorption rate through human epidermis over the first 10 hours was 0.035 µg/cm^2/h, which slightly increased to a constant rate of 0.041 µg/cm^2/h between 2 and 24 hours. Absorption was faster through the rat epidermis than through the human epidermis. It was about 5 times faster with the concentrate and up to 8 times faster with the spray dilution. In the rat, the mean absorption rate of the concentrate was essentially constant throughout the exposure period (1.03 µg/cm^2/h). For the diluted Fipronil formulation, it was 0.275 µg/cm^2/h during the first 10 hours, and averaged 0.185 µg/cm^2/h over the whole 24 hours.	X
	During a typical 6-10 hour working day, the mean quantity of Fipronil absorbed from the concentrate through human epidermis was 4.3-4.6 µg/cm^2 and 10.6-13.4 µg/cm^2 through the rat (corresponding to 0.14-0.15% and 0.34-0.43% of applied dose, respectively). For the dilution, whilst the amount absorbed was lower (0.13-0.29 and 3.8-4.3 µg/cm^2, respectively), it represented a higher percentage of the applied dose (0.22-0.49 % and 6.4-7.1% in human and rat epidermis, respectively). Following 24-hours of exposure to the concentrate, 4.8 and 25.6 µg/cm^2 of Fipronil (0.15% and 0.82% of the applied dose) had been absorbed by human and rat epidermis, respectively. 0.89 and 6.2 µg/cm^2 of the dilution (corresponding to 1.5 and 10.3% of the applied dose) penetrated through human and rat epidermis, respectively within the 24-h exposure period. Comparison of these data indicate that the diluted Fipronil dose was more readily absorbed through rat or human epidermis than the higher concentrated Fipronil dose.	X
5.3 Conclusion	Absorption of Fipronil through the human epidermis varied from slow for the concentrate to very slow for the spray dilution of a 300 g/l EC formulation (BAS 350 43 I). The data predicted that dermal absorption from either the concentrate or dilution would be minimal during a typical exposure period (6-10 hours) and that the majority of any Fipronil contacting human skin would be removed during normal washing procedures. Furthermore, as a model for predicting dermal absorption of Fipronil, the rat overestimates absorption through human skin.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. This is not considered to lead to underestimation of fipronil's dermal absorption potential, since the presence of formulants usually lead to enhanced absorption. Furthermore, additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations.	

Table A6.2.6-1 Summary of absorption of Fipronil through human and rat epidermis following application of the concentrate and a 1:50 aqueous dilution of a 300 g/l EC formulation

Time (hours)	Concentrate (300 g/l)		Dilution (6 g/l)	
	Rat	Human	Rat	Human
Mean absorption rates (µg/cm²/h)				
1-24	~~1.03~~ nd	0.215~~a~~[a]	nd	nd
1-10	~~Nd~~ 1.03	nd	0.275	0.035
2-24	nd	nd	0.185	0.041
Mean amount absorbed (µg/cm²)				
0–6	10.6	4.3	3.8	0.13
0–8	11.7	4.4	4.1	0.22
0–10	13.4	4.6	4.3	0.29
0–24	25.6	4.8	6.2	0.89
% of dose absorbed				
0–6	0.34	0.14	6.4	0.22
0–8	0.37	0.14	6.9	0.36
0–10	0.43	0.15	7.1	0.49
0–24	0.82	0.15	10.3	1.5

[a] Fipronil absorption was only noted in 1/5 cells tested; the LOQ value of 0.186 µg/cm²/h was substituted for the other 4 cells to allow calculation of the mean.
nd Not determined/measured

X

Table A6.2.6-2 Mass balance and distribution of Fipronil following 24-h application of the concentrate and an aqueous 1:50 dilution of a 300 g/l EC formulation to human and rat epidermis (mean % of dose recovered)

	Concentrate (300 g/l)		Dilution (6 g/l)	
	Rat	Human	Rat	Human
Spreader	1.76	12.4	0.50	10.8
Donor chamber	20.0	27.3	6.22	10.5
Skin wash	82.9	77.0	31.3	66.8
Epidermis	7.0	1.07	50.8	7.76
Absorbed	0.75	0.15	10.3	1.48
Total	112	118	99.1	97.3

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1.2.2 Purity/3.1.2.3 Stability: 28.2% of Fipronil. The purity and stability of the spray dilution are the responsibility of the sponsor. 3.2.6 Number of cells per group: *Concentrate: 6~~/species~~* cells for rat and 5 cells for human epidermis.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: Mass balance and distribution (see Table A6.2.6-2) Only the quantity of Fipronil in the receptor fluid was considered to be absorbed. Amounts from the spreader, donor chamber, rinsings and the epidermis itself were considered unavailable for absorption. Fipronil in the epidermis was also deemed unabsorbed since it will most likely be lost *in vivo* by desquamation. In the human epidermis the majority of the applied dose (77% and 67 % for concentrate and dilution, respectively) was washed off during decontamination. Similarly, in the rat, 83% and 31% of the applied doses of the concentrate and dilution, respectively, was released by decontamination. For human epidermis, about 40% and 21% of the concentrate and dilution, respectively, were deemed unavailable whilst the corresponding values for rat epidermis were slightly lower (22% and 7%, respectively). Following application of the concentrate and dilution, the amounts of Fipronil in the human epidermis (1.07% and 7.76%, respectively) were markedly lower than corresponding values for the rat epidermis (7% and 51% respectively). The amount of Fipronil absorbed through the human epidermis during the 24-hour exposure period was 0.15% of the applied dose of the concentrate and 1.5% for the dilution. In the rat, 0.75% and 10.3% of the applied dose of the concentrate and dilution, respectively, was absorbed. The quantity of fipronil in the receptor fluid was considered to be absorbed directly and the quantity in the epidermis was considered to be available to be absorbed, indirectly. In the OECD 428 guideline, it is written that "the test substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid values alone"
Conclusion	Agree with applicant's version.
Reliability	2 –The study was not made with the pure substance but with a formulation.
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks

Section A6.2 **Annex Point IIA6.2**	***In vitro* dermal penetration study with Fipronil 50 g/l SC formulation**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.2/07** XXXX. Fipronil: In vitro absorption from a 50 g/l SC formulation through human and rat epidermis 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 1.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Not available at the time the study was conducted; in general compliant to OECD 428 (adopted 2004)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	a) Regent 50 SC (= EXP 60658C): Commercial SC formulation with nominal 50 g/l Fipronil (containing ^{14}C-radiolabelled Fipronil) Please see IIIA 6.2 - BCI b) 1:100 (v/v) aqueous dilution of Regent 50 SC formulation with nominal 0.5 g/l Fipronil (containing ^{14}C-radiolabelled Fipronil)	
3.1.1 Lot/Batch number	a1) 1881 FD 96; Radiolabelled fipronil ref. no. 4949 a2) OP1923 FD 97; Radiolabelled fipronil ref. no. 4989 b) 1882 FD 96; Radiolabelled fipronil ref. no. 4956	**X**
3.1.2 Specification	a1) 1881 FD 96: 49.7 g Fipronil/L a2) OP1923 FD 97: 49.6 g Fipronil/L b) 1882 FD 96: nominal 0.5 g Fipronil/L	
3.1.2.1 Description	a) Opaque grey/white liquid b) White liquid	
3.1.2.2 Purity	Fipronil content in formulation: 49.6 / 49.7 g/l or 0.5 g/l	**X**
3.1.2.3 Stability	Stable for the duration of the test	**X**
3.1.2.4 Radiolabelling	^{14}C- label uniform at phenyl moiety	
3.2 Test system		
3.2.1 Rat epidermis	Epidermis were prepared from male Wistar rats aged ca.28 days. Subcutaneous fat was removed, Skins wer soaked for ca. 20 hours in 1.5 M Sodium bromide, washed and the epidermis carefully peeled from the dermis	
3.2.2 Human epidermis	Underlying muscle and fat were removed from human whole skin samples. The skin samples were immersed in water at 60°C for 40-45 seconds and the epidermis separated from the dermis.	
3.2.4 Chamber	Horizontal glass diffusion cells, static conditions, receptor chamber maintained at 30 ± 1°C	

Section A6.2 **Annex Point IIA6.2**	***In vitro* dermal penetration study with Fipronil 50 g/l SC formulation**	
3.2.5 Membrane integrity check	Yes The integrity of the epidermal membranes was determined by measurement of their electrical resistance. Membranes with a measured resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used.	
3.2.6 Number of cells per group	Concentrate: 6/rat and 7/human epidermis Dilution: 5/rat and 6/human epidermis	**X**
3.2.7 Reference substances	No	
3.2.8 Receptor fluid	50% v/v ethanol in distilled water	
3.2.9 Quantification of radioactivity	Liquid Scintillation Counting	
3.2.9 Endpoints	Skin permeation (flux), % applied dose penetrated, mass balance	
3.3 Administration/ Exposure		
3.3.1 Preparation of test substance	The samples were tested as supplied.	
3.3.2 Concentration of test substance	Concentrate: 49.7 or 49.6 g/l Dilution: 0.5 g/l	
3.3.3 Specific activity of test substance	Concentrate: a1) 3 MBq / 2.47 g; a2) 4.66 MBq / 3.26 g Dilution: 1.55 MBq / 3.47 g	
3.3.4 Volume applied	10 µl/cm²	
3.3.5 Area of skin	2.54 cm²	
3.3.6 Exposure period	24 h	
3.3.7 Sampling time	Not specifically mentioned in the report. Time-sbsorption diagrams indicate that samples were obtained at the following time points post application: 1, 2, 3, 4, 6, 8, 10, 12, 18, 20, 22, 24 h.	
4.1 Penetration rate Flux (µg/cm²/h)	**4. RESULTS AND DISCUSSION** (See Table A6.2.7-1) Within 24 hours of exposure, the following mean fluxes could be calculated through rat / human epidermis: Undiluted formulation: 2.99 / 0.044–0.052[a] µg/cm²/h 1:100 aqueous dilution: 0.056 / 0.005 µg/cm²/h [a] Fipronil absorption of the concentrate was only noted in 2/7 cells tested for the first 10 hours; the LOQ value of 0.05 µg/cm²/h was substituted for the other 5 cells to allow calculation of the mean.	**X**

Section A6.2 **Annex Point IIA6.2**	***In vitro* dermal penetration study with Fipronil 50 g/l SC formulation**	
4.2 % radiolabel permeated	(See Table A6.2.7-1) Within 6–10 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 3.2–5.5% / <0.1–0.15 % 1:100 diluted formulation: 14.5–20.2% / 0.82–1.2% Within 24 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / human epidermis were recovered in the receptor chamber: Undiluted formulation: 15% / 0.15 % 1:100 diluted formulation: 34.5% / 2.7 %	X
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Radiolabelled [^{14}C] samples of the concentrated 50 g/l of Fipronil SC formulation and an aqueous 1:100 v/v spray strength dilution of this formulation were used to assess the absorption of Fipronil *in vitro* through human and rat epidermis. Human and rat epidermal skin, excluding the dermis and underlying fat/muscle, with an electrical resistance of >10 kΩ (human) or >2.5 kΩ (rat) were used. Samples of each epidermis were exposed to the concentrate and spray dilution as follows. A sample of epidermis was placed on a support grid in the chamber of a glass diffusion cell. 10 µl/cm² of the concentrate or dilution was applied to approx. 2.54 cm^2 of the skin preparation. A recorded volume of receptor fluid (50%v/v ethanol in distilled water) was put in the receptor beneath the mounted skin sample and the cell placed in a water bath maintained at 30 ± 1°C. The epidermis was unoccluded for the 24-hour exposure period to simulate potential human dermal exposure during normal use. At recorded intervals pre-and post-dosing, samples (0.1 ml) of the chamber fluid were taken and replaced by fresh fluid. The radioactivity in each sample was quantified by liquid scintillation to determine the amount of Fipronil. At the end of the exposure period, each epidermis sample was carefully decontaminated by washing and digested. Radioactivity in the washings and the digested epidermis were quantified and the mass-balance of the various fractions and the distribution of radiolabel were determined.	X
5.2 Results and discussion	<u>Absorption</u> (see Table A6.2.7-1) Absorption of both the concentrate and spray dilution was much faster through human epidermis than through rat tissue (70- and 23-fold, respectively).	X

Section A6.2 **Annex Point IIA6.2**	***In vitro* dermal penetration study with Fipronil 50 g/l SC formulation**	
	Absorption of Fipronil from the concentrate through the human epidermis was not detected until 10 hours after exposure, giving a mean rate of 0.052 µg/cm^2/h over this period. This was derived using the LOQ (0.05 µg/cm^2/h) as the theoretical maximum value for the 5 of 7 cells where no absorption was observed. With respect to the spray dilution, the rate of absorption was essentially constant over 24 hours (0.005 µg Fipronil/cm^2/h).	**X**
	In rat epidermis, the mean rate of absorption from the concentrate was essentially constant throughout exposure (2.99 µg/cm^2/h). With respect to the spray dilution, absorption during a working day (6-10 hours) was 0.088 µg/cm^2/h. It was fastest during the first 6 hours (0.115 µg/cm^2/h) whilst a constant rate of 0.056 µg/cm^2/h was maintained over 4-24 hours.	
	In terms of the amount of Fipronil absorbed over a typical working day period (6-10 hours), values for the concentrate were between <0.5-0.52 µg/cm^2 (<0.1 to 0.1% of the applied dose) through human epidermis and 16.0-27.2 µg/cm^2 (3.2-5.5% of the applied dose) for rat epidermis. Application of the spray dilution resulted in lower quantities of Fipronil being absorbed (0.041-0.059 µg/cm^2/h for human tissue and 0.72-1.01 µg/cm^2 for rat epidermis) but these corresponded to higher percentages of the applied dose (0.82-1.2% and 14.5-20.2% for human and rat epidermis, respectively).	
	Following 24-hours of exposure to the concentrate, 0.77 and 72 µg/cm^2 of Fipronil (0.15% and 15% of the applied dose) had been absorbed from the concentrate by human and rat epidermis, respectively. From the spray dilution, 0.134 and 1.73 µg/cm^2 were absorbed, respectively (2.7% and 34.5% of the respective applied dose). Therefore, a higher proportion of the dilution was absorbed by both human and rat epidermis compared with the concentrate.	
	<u>Mass balance and distribution</u> (see Table A6.2.7-2) Only the quantity of Fipronil in the receptor fluid was considered to be absorbed. Amounts from the spreader, donor chamber, rinsings and the epidermis itself were considered unavailable for absorption. Fipronil in the epidermis was deemed unabsorbed since it will most likely be lost *in vivo* by desquamation.	**X**
	In the human epidermis, the majority of the applied dose (about 97% and 83% for concentrate and dilution, respectively) was washed off during decontamination. In the rat, 37 and 60% of the applied doses of the concentrate and dilution, respectively, were released by decontamination. For human epidermis, about 11 and 25% of the concentrate and dilution were deemed unavailable. Similar values were obtained for rat epidermis (15 and 16%, respectively). Following decontamination 24 hours post-application of the concentrate and spray dilution, the amounts of Fipronil in the human epidermis were 0.95% and 8.7%, respectively, which were similar to those for the rat epidermis (about 7% for both the concentrate and spray dilution).	**X**

Section A6.2 **Annex Point IIA6.2**	***In vitro* dermal penetration study with Fipronil 50 g/l SC formulation**	
	The mean quantity of Fipronil absorbed through human epidermis during the 24-hour exposure period was 0.15% of the applied dose of the concentrate and 2.7% for the spray dilution, respectively. In the rat, 15% and 35% of the applied dose of the concentrate and dilution, respectively, was absorbed.	
5.3 Conclusion	Absorption of Fipronil through human epidermis from a 50 g/l SC formulation varied from very slow for the concentrate, to extremely slow for the spray strength dilution compared with the absorption rates of other penetrants measured using this method. The data predicted that during a typical exposure period (6-10 hours) dermal absorption from the concentrate or dilution would be minimal, and that the majority of any Fipronil in contact with human skin would be removed during normal washing procedures. Furthermore, as a model for the predicting dermal absorption of this compound, the rat greatly overestimates absorption through human skin.	
5.3.1 Reliability	2	
5.3.2 Deficiencies	The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. This is not considered to lead to underestimation of fipronil's dermal absorption potential, since the presence of formulants usually lead to enhanced absorption. Furthermore, additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations.	

Table A6.2.7-1 Summary of absorption of Fipronil through human and rat epidermis following application of the concentrate and a 1:100 aqueous dilution of a 50 g/l SC formulation

Time (hours)	Concentrate (50 g/l)		Dilution (0.5 g/l)	
	Rat	Human	Rat	Human
Mean absorption rates (µg/cm²/h)				
1-6	nd	nd	0.115	nd
1-10	nd	0.052a	0.088	nd
10-24	nd	0.044a	nd	nd
1-24	2.99	nd	nd	0.005
4-24	nd	nd	0.056	nd
Mean amount absorbed (µg/cm²)				
0–6	16.0	<0.5	0.72	0.041
0–8	21.9	<0.5	0.82	0.050
0–10	27.2	0.52	1.01	0.059
0–24	72.0	0.77	1.73	0.134
% of dose absorbed				
0–6	3.2	<0.1	14.5	0.82
0–8	4.4	<0.1	16.3	1.0
0–10	5.5	0.1	20.2	1.2
0–24	15	0.15	34.5	2.7

[a] Fipronil absorption was only noted in ~~2/7~~ 2/6 cells tested; the LOQ value of 0.05 µg/cm²/h was substituted for the other ~~5~~ 4 cells to allow calculation of the mean.

nd Not determined/measured

X

Table A6.2.7-2 Mass balance and distribution of Fipronil following application of the concentrate and an aqueous 1:100 dilution of a 50 g/l SC formulation to human and rat epidermis (mean % of dose recovered after 24-h exposure)

	Concentrate (50 g/l)		Dilution (0.5 g/l)	
	Rat	Human	Rat	Human
Spreader	8.35	7.41	6.99	19.1
Donor chamber	6.62	3.32	9.17	6.28
Skin wash	36.5	107 (96.6)	60.1	82.8
Epidermis	7.09	0.95	7.29	8.68
Absorbed	14.5	0.154	34.6	2.68
Total	73.0	119 (108)	118	120

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1.1 Lot/Batch number: *OP1923 FD 97; Radiolabelled fipronil ref. no. 4989* was used for exposure to rat epidermis because the sample 1881 FD 96 was not sufficiently stable to allow the experiment for its application to rat skin. 3.1.2.2 Purity/3.1.2.3 Stability: The purity and stability of the spray dilution are the responsibility of the sponsor. 3.2.6 Number of cells per group: *Concentrate: 6/rat and ~~7/human~~* 6/human *epidermis*
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Penetration rate flux: *Undiluted formulation: 2.99 / 0.044[b]–0.052[a] µg/cm²/h* *[a] Fipronil absorption of the concentrate was only noted in ~~2/7~~ 2/6 cells tested for the first 10 hours; the LOQ value of 0.05 µg/cm²/h was substituted for the other ~~5~~ 4 cells to allow calculation of the mean.* [b] Mean absorption rate in the 10-24 time period. 4.2 % radiolabel permeated: *Undiluted formulation: 3.2–5.5% / ~~<0.1–0.15 %~~* <0.1-0.10%
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *and an aqueous 1:100 v/v spray strength dilution of this formulation* containing nominal 0.5 g/l Fipronil. 5.2 Results and discussion: ~~*Absorption of both the concentrate and spray dilution was much faster through human epidermis than through rat tissue (70- and 23-fold, respectively).*~~ Absorption of both the concentrate and spray dilution through rat epidermis was much faster than through human epidermis (70- and 23-fold, respectively). ~~*5 of 7 cells*~~ 4 of 6 cells. Mass balance and distribution: ~~*Only the quantity of Fipronil in the receptor fluid was considered to be absorbed. Amounts from the spreader, donor chamber, rinsings and the epidermis itself were considered unavailable for absorption. Fipronil in the epidermis was deemed unabsorbed since it will most likely be lost in vivo by desquamation.*~~ The quantity of fipronil in the receptor fluid was considered to be absorbed directly and the quantity in the epidermis was considered to be available to be absorbed, indirectly. In the OECD 428 guideline, it is written that "the test substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid values alone" *In the human epidermis, the majority of the applied dose (about ~~97%~~ 107% and 83% for concentrate and dilution, respectively) was washed off during decontamination.*

Reliability **Acceptability** **Remarks**	2 –The study was not made with the pure substance but with a formulation. acceptable
Date **Results and discussion** **Conclusion** **Reliability** **Acceptability** **Remarks**	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil 200 g/l SC formulation**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.2/08** XXXX In vitro skin permeability of M&B 46030. XXXX. (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 1.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Not available at the time the study was conducted; in general compliant to OECD 428 (adopted 2004)	
2.2 GLP	No	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	a) BAS 350 61 I (= EXP 60145A = Regent 200 SC) blank formulation; Please see IIIA 6.2 - BCI b) (^{14}C)-fipronil c) Unlabelled fipronil	**X**
3.1.1 Lot/Batch number	Radiolabelled fipronil: batch no. GHS 634A	
3.1.2 Specification	BAS 350 61 I: contains 200 g/l Fipronil	**X**
3.1.2.1 Description	Off-white solid powder	
3.1.2.2 Purity	Radiolabelled Fipronil: 98.7% Fipronil content in Formulation: 200 g/l	
3.1.2.3 Stability	stable	
3.1.2.4 Radiolabelling	^{14}C- label uniform at phenyl moiety	
3.2 Test system		
3.2.1 Rat epidermis	300 µm thick dermatomized skin (epidermis) was prepared from dorsal and flank regions of female rats aged 21-28 days	**X**
3.2.2 Rabbit epidermis	300 µm thick dermatomized skin (epidermis) was prepared from dorsal and flank regions of female NZW rabbits, 2.0-2.5 kg bw	**X**
3.2.3 Human epidermis	Epidermis samples were prepared from abdominal skin of a female human donor (autopsy material), The epidermal membrane was obtained via heat separation following immersion of whole skin at 60 for about 45 sec.	**X**
3.2.4 Chamber	Horizontal glass diffusion cells, static conditions, receptor chamber maintained at 37 ± 0.5°C	
3.2.5 Membrane integrity check	Yes permeability measured on day 1 with tritiated water	
3.2.6 Number of cells per group	5/dose/species	**X**
3.2.7 Reference substances	Yes Hydrocortisone and testosterone	
3.2.8 Receptor fluid	50% aqueous ethanol	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil 200 g/l SC formulation**	
3.2.9 Quantification of radioactivity	Liquid Scintillation Counting	
3.2.9 Endpoints	Skin permeation (flux), % applied dose penetrated	
3.3 Administration/ Exposure		
3.3.1 Preparation of test substance	Radiolabelled and unlabelled fipronil formulated in blank formulation to generate 20% stock formulation.	**X**
3.3.2 Concentration of test substance	Neat formulation (containing 200 g Fipronil/l); 1:50 (4 g/l) or 1:1000 (0.2 g/l) dilution in distilled water	
3.3.3 Specific activity of test substance	45.1 µCi/mg	
3.3.4 Volume applied	100 µl/cm²	
3.3.5 Area of skin	0.75 – 2.75 cm² (exact area measured for each donor cell)	
3.3.6 Exposure period	8 and 24 h	
3.3.7 Sampling time	1, 2, 3, 4, 6, 8 and 24 h after initiation	
	4. RESULTS AND DISCUSSION	
4.1 Penetration rate Flux (µg/cm²/h)	(See Table A6.2.8-1) Within 8 hours of exposure, the following mean fluxes could be calculated through rat / rabbit / human epidermis: Undiluted formulation: 1.92 / 1.68 / 0.18 µg/cm²/h 1:50 diluted formulation: 0.07 / 0.33 / 0.03 µg/cm²/h 1:1000 diluted formulation: 0.02 / 0.35 / 0.02 µg/cm²/h Within 24 hours of exposure, the following mean fluxes could be calculated through rat / rabbit / human epidermis: Undiluted formulation: 9.92 / 6.34 / 0.42 µg/cm²/h 1:50 diluted formulation: 0.99 / 0.80 / 0.03 µg/cm²/h 1:1000 diluted formulation: 0.24 / 0.42 / 0.11 µg/cm²/h	
4.2 % radiolabel permeated	(See Table A6.2.8-1) Within 8 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / rabbit /human epidermis and were recovered in the receptor chamber: Undiluted formulation: 0.08 / 0.07 / 0.01 % 1:50 diluted formulation: 0.14 / 0.67 / 0.07 % 1:1000 diluted formulation: 0.90 / 13.9 / 0.98 % Within 24 hours of exposure, the following mean percentages of administered radiolabel permeated through rat / rabbit /human epidermis and were recovered in the receptor chamber: Undiluted formulation: 1.19 / 0.76 / 0.05 % 1:50 diluted formulation: 5.96 / 4.80 / 0.18 % 1:1000 diluted formulation: 24.2 / 50.1 / 12.9 %	

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil 200 g/l SC formulation**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Radiolabelled [^{14}C]-Fipronil, mixed with the respective unlabelled material was formulated in the blank suspension concentrate (SC) formulation BAS 350 61 I (*syn.* EXP 60145A or Regent 200 SC) to generate a Fipronil concentrated product of 200 g/l. The same procedure was applied with reference compounds [^{14}C]-Hydrocortisone (specific activity: 154 µCi/mg, purity: 97.1%) and [^{14}C]-Testosterone (specific activity: 196 µCi/mg, purity: 96.2%). Absorption rates through human, rabbit and rat epidermis of the neat Fipronil formulation (200 g/l) and two aqueous dilutions (containing 4.0 g/l and 0.2 g/l) were determined together with the absorption rates of Testosterone and Hydrocortisone (both at 4.0 g/l as aqueous dilutions of blank BAS 350 61 I). Rabbit and rat epidermal skin membranes, excluding the dermis and underlying fat/muscle, were prepared by freezing the dermal side of the membrane to a steel plate and dermatoming it to approximately 300 µm. Human epidermal skin membranes were prepared by heating the skin in water at 60° C for 45 seconds and then peeling away the dermis. The prepared epidermal membranes were placed as a barrier between the two halves of horizontal type glass diffusion cells. The area available for diffusion was approximately 0.75-2.75 cm^2 and the measured receptor chamber volume varied from 2.2-9.8 ml. The diffusion cells were maintained at 37.0 ± 0.5^{o}C in a water bath and the receptor chamber contents agitated continuously. The integrity of the membranes was checked on Day 1 by measuring their permeability to tritiated water. On Day 2, aliquots of formulated solutions (100 µl/cm^2) of radiolabelled materials were placed on the skin surface and the donor chamber was covered to prevent evaporative loss. Radiolabelled Fipronil was used either neat (200 g/l), or diluted to 1:50, or 1:1000 (i.e. 4.0 or 0.2 g/l). Aqueous dilutions of formulated radiolabelled testosterone or hydrocortisone were used (4.0 g/l). Samples (200 µl) of the bulk donor solution were taken at time point 0. Similar sized samples were taken from the receptor chamber 1, 2, 3, 4, 6, 8 and 24 hours after dosing. All samples were added to 5 ml liquid scintillation fluid and the radioactivity in each sample quantified by liquid scintillation to determine the amount of Fipronil, hydrocortisone or testosterone present.	
5.2 Results and discussion	(See Table 6.2.8-1) The rate of absorption of both the neat and 1:50 dilution of Fipronil (200 g/l and 4 g/l) was faster through rat and rabbit epidermis than through human epidermis. Furthermore, the degree of absorption was enhanced by dilution. Therefore, the amount of Fipronil absorbed was highest at the most diluted concentration (0.2 g/l). The penetration rate of Fipronil through human skin, at an equivalent concentration, is an order of magnitude less than that of Hydrocortisone. The latter compound is considered to be a relatively poor penetrant.	**X**

Section A6.2 **Annex Point IIA, VI.6.2**	***In vitro* dermal penetration study with Fipronil 200 g/l SC formulation**

5.3 Conclusion	Dermal absorption of Fipronil was faster through rat and rabbit epidermis than through human epidermis, the rate and extent of absorption was highest for the most dilute concentration (0.2 g/l). Fipronil was considered to be a slow dermal penetrant compared with Testosterone and Hydrocortisone.
5.3.1 Reliability	2
5.3.2 Deficiencies	Not a GLP study and conducted before validated Test Guidelines were available. The test was performed with radiolabelled fipronil in a commercial formulation rather than with only the active material. This is not considered to lead to underestimation of fipronil's dermal absorption potential, since the presence of formulants usually lead to enhanced absorption. Furthermore, additional in-vitro dermal penetration assays with various other fipronil formulations have confirmed the very low dermal penetration potential of fipronil through rat and human epidermis preparations.

Table 6.2.8-1 Absorption of [^{14}C]-labelled Fipronil, testosterone and hydrocortisone, formulated in Regent 200 SC through rat, rabbit and human epidermis

Species	Time (hours)	Fipronil			Testosterone	Hydrocortisone
		200 g/l	4.0 g/l	0.2 g/l	4.0 g/l	4.0 g/l
Mean flux (µg/cm^2/h)						
Rat	8	1.92	0.07	0.02	7.72	3.19
	24	9.92	0.99	0.24	11.20	6.44
Rabbit	8	1.68	0.33	0.35	2.94	3.02
	24	6.34	0.80	0.42	4.90	2.79
Human	8	0.18	0.03	0.02	0.17	0.29
	24	0.42	0.03	0.11	0.37	0.41
% dose absorbed						
Rat	8	0.08	0.14	0.90	15.44	6.38
	24	1.19	5.96	24.15	67.19	38.67
Rabbit	8	0.07	0.67	13.88	5.89	6.05
	24	0.76	4.80	50.06	29.43	16.71
Human	8	0.01	0.07	0.98	0.33	0.58
	24	0.05	0.18	12.89	2.24	2.47

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *XXXX*. 3.1 Test material: ~~*a) BAS 350 61 I (= EXP 60145A = Regent 200 SC) blank formulation; c) Unlabelled fipronil*~~ Neat formulation containing 200 g Fipronil/l and two dilutions 1:50 (4 g/l) and 1:1000 (0.2 g/l). 3.1.2 Specification: ~~*BAS 350 61 I:*~~ The formulation *contains 200 g/l Fipronil*, two aqueous dilutions contain 4 g/l or 0.2 g/l active. 3.2.1 Rat epidermis: *300 µm thick dermatomized skin (epidermis) was prepared from dorsal and flank regions of female* Sprague-Dawley CD *rats aged 21-28 days* Any adherent subcutaneous fat was removed by blunt dissection. 3.2.2 Rabbit epidermis: Any adherent subcutaneous fat was removed by blunt dissection. 3.2.3 Human epidermis: *at 60*°C *for about 45 sec*. The epidermis was peeled away from the dermis. 3.2.6 Number of cells per group: *5/dose/species* except for the rat skin treated with 0.2 g/l and for the human skin treated with 4.0 g/l (n=4). 3.3.1 Preparation of test substance: *Radiolabelled and unlabelled fipronil formulated in blank formulation to generate 20% stock formulation.* 24 mg of radiolabelled fipronil was made up to 2g using unlabelled compound. This mixture was formulated in the blank formulation EXP 60145A to generate a concentration of 20% w/w.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion The quantity of fipronil in the receptor fluid was considered to be absorbed directly and the quantity in the epidermis was considered to be available to be absorbed, indirectly. In the OECD 428 guideline, it is written that "the test substance remaining in the skin should be considered as absorbed unless it can be demonstrated that absorption can be determined from receptor fluid values alone"
Reliability	2 –The study was not made with the pure substance but with a formulation.
Acceptability	acceptable
Remarks	The formulation of EXP 60145A is not submitted.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	

Reliability **Acceptability** **Remarks**

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.2/09** XXXX. Fipronil: Tissue Kinetic study in the rat. XXXX (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 1.	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes: EU 94/79/EEC OECD 417 (1984) JMAFF NohSan no 4200 (1985)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	**X**
3.1.1 Lot/Batch number	GHS 826	
3.1.2 Specification	As given in Section 2	**X**
3.1.2.1 Description	None given	
3.1.2.2 Purity	Radiopurity ≥99%	
3.1.2.3 Stability	stable	**X**
3.1.2.4 Radiolabelling	^{14}C (phenyl ring uniformly labeled)	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Charles River Crl:CD	**X**
3.2.3 Source	XXXX	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Young adult 166.6 – 309.02 g	
3.2.6 Number of animals per group	68 (34 male and 34 female)	**X**
3.2.7 Control animals	No	
3.3 Administration/ Exposure	Oral	
3.3.1 Type	Gavage	
3.3.2 Dose levels	4 and 40 mg/kg See Table A6.2.9-1	
3.3.3 Preparation of test substance	Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80	**X**
3.3.4 Total volume applied	1g dose per 250g rat bodyweight at the required active ingredient level	
3.3.5 Post-exposure period	168 hours	**X**

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**	
	4. APPLICANT'S SUMMARY AND CONCLUSION	
4.1 Materials and methods		
4.1.1 Treatment	Groups of 34 rats/sex were either given a single nominal dose of 4 or 40 mg/kg bodyweight [^{14}C]-fipronil by gavage. (Blood pharmacokinetics , Groups A and B and Tissue Distribution Kinetics Groups C and D). Fipronil was administered as a suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80	X
4.1.2 Sampling	Groups A and B: Blood samples (approximately 0.1 to 0.2g) were collected in heparinised glass capillaries prior to dosing and at approximately 0.5, 1, 2, 3, 4, 6, 8 and 24 hours after dosing and at twenty four hourly intervals thereafter until approximately 336 hours post-dosing.	
	Groups C and D: Following administration of [^{14}C]-Fipronil, animals were exsanguinated under anesthesia at time periods corresponding to 1/2T_{max} (abs), T_{max}, 1/2T_{max} (elim) and 168 h: (Table A6.2.9-2). The liver, kidneys, heart, lungs, brain, spleen pancreas, muscle, abdominal fat, ovaries, testes, gastrointestinal tract plus contents, stomach plus contents, bone (femur) and marrow, adrenals, uterus, thyroid and the skin and fur were removed from each animal after exsanguination. The residual carcass was also retained for analysis. Plasma was prepared from cardiac blood samples by centrifugation.	X
4.1.3 Radioassay	The amounts of radioactivity in the various samples were determined by liquid scintillation counting. Samples were counted for 10 minutes or 2 sigma % in an appropriate scintillation cocktail using a counter with on-line computing facilities in which quenching effects were determined using an external standard and spectral quench parameter (tie) method. Efficiency correlation curves were prepared for each scintillation cocktail and were regularly checked by the use of [^{14}C}-n-hexane standards. The scintillation counter was recalibrated when a deviation of greater than 2% was observed when counting quality control standards.	X
4.1 Results		
Blood pharmacokinetic parameters	See Table A6.2.9-2 There was no apparent sex difference in blood pharmacokinetic parameters following a single oral administration of [^{14}C]-fipronil to male and female rats at 40 mg/kg bodyweight. Peaks (C_{max}) in mean blood radioactivity of 6.684 ± 1.466 µg equiv/g (males) and 7.584 ± 0.322 µg equiv/g (females) were attained at 33.6 and 38.4 hours respectively. Concentrations thereafter fell up to 336 hours post-dose (ca. 4% of C_{max} at 336h). During the absorption phase, estimated ½C_{max} was achieved in male and female rats at 3 and 4 hours respectively. The elimination phase estimated ½C_{max} was achieved at 77 and 78 hours post-dose in the males and females respectively. The elimination half-lives were calculated to be 135.4 ± 15.8 hours (males) and 170.6 ± 27.3 hours (females).	

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**
Distribution	Following a single oral administration of [^{14}C]-fipronil to male and female rats at 4 mg/kg bodyweight, peaks (C_{max}) in mean blood radioactivity of 0.519± 0.058 µg equiv/g (males) and 0.394 ± 0.084 µg equiv/g (females) were attained at 4.8 and 6.2 hours respectively. Concentrations thereafter fell up to 336 hours post-dose. Levels declined more slowly than was observed for the high dose and were ca 20% of C_{max} at 336 hours. During the absorption phase, estimated ½C_{max} was achieved in male and female rats at 0.75 and 0.83 hours respectively. The elimination phase estimated ½C_{max} was achieved at 96 and 94 hours post-dose in the males and females respectively. The elimination half-lives were calculated to be 182.5 ± 22.4 hours (males) and 245.2 ± 34.8 hours (females). See Tables 6.2.9-3 and 4 At 3 hours post-dose following administration of [^{14}C]-fipronil at 40 mg/kg bodyweight to male rats, the highest mean levels of radioactivity were present in gastrointestinal tract plus contents and in the stomach plus contents (421.88 ± 41.15 and 380.82 ± 61.31 µg equiv/g), fat (68.63 ± 13.42 µg equiv/g), adrenals (34.49 ± 3.48 µg equiv/g), pancreas (31.46 ± 17.07 µg equiv/g), liver (30.96 ± 5.41 µg equiv/g) and thyroids (25.14 ± 8.70 µg equiv/g). Mean levels of radioactivity in the remaining tissues were between 16.95 to 2.09 µg equiv/g (skin, fur and cardiac blood). No tissues presented mean levels of radioactivity below the limit of detection At 33.6 hours post-dose, levels of radioactivity had increased in fat (228.61 ± 55.13.µg equiv/g), adrenals (53.93 ± 20.35 µg equiv/g) and in skin and fur (29.26 ± 2.62 µg equiv/g) above the levels detected at 3 hours post-dose. However, in the remaining tissues radioactivity levels had remained constant or had fallen. Other than fat, highest mean levels were found in gastrointestinal tract (73.26 ± 22.98 µg equiv/g), stomach (64.41 ± 48.15 µg equiv/g), pancreas (37.69 ± 8.01µg equiv/g), liver (35.68 ± 7.44 µg equiv/g) and thyroids (29.37 ± 17.81 µg equiv/g). At 77 hours post-dose, levels of radioactivity had fallen in all tissues. Highest mean levels were in fat (114.85 ± 5.15 µg equiv/g) and in gastrointestinal tract (49.11 ± 7.23 µg equiv/g). Mean levels of radioactivity in the organs of metabolism and excretion, liver and kidney were relatively low: 16.88 ± 1.38 µg equiv/g and 8.84 ± 0.09 µg equiv/g respectively. By 168 hours post-dose levels of radioactivity had continued to fall. Highest mean levels were in fat (32.05 ± 3.03 µg equiv/g) , adrenals (15.79 ± 2.84 µg equiv/g) and thyroids (10.48 ± 3.13 µg equiv/g).

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**	
	In female rats the distribution of radioactivity with time was essentially similar to that obtained in male rats. At 4 hours post-dose, highest levels of radioactivity were located in gastrointestinal tract plus contents and in stomach plus contents (359.97 ± 77.27 µg equiv/g and 184.86 ± 132.98 µg equiv/g), fat (79.88 ± 14.43 µg equiv/g), adrenals (39.15 ± 9.76 µg equiv/g), liver (32.14 ± 3.59 µg equiv/g), pancreas (26.11 ±1.66 µg equiv/g) and skin and fur (19.82 ± 1.01 µg equiv/g). The only sex difference was observed for the gonads where the concentrations in the ovaries (20.04 ± 4.32 µg equiv/g) were markedly higher than those obtained in testes (6.05 ± 0.55 µg equiv/g). Mean levels of radioactivity in the remaining tissues were between 17.72 to 2.16 µg equiv/g (uterus and cardiac blood). No tissues presented mean levels of radioactivity below the limit of detection By 38.4 hours post-dose, mean levels of radioactivity had increased in fat (201.24 ± 10.56µg equiv/g), ovaries (43.97 ± 3.75 µg equiv/g) , uterus (30.49 ± 5.83 µg equiv/g) and skin (29.43 ± 3.83µg equiv/g) but had fallen in the gastrointestinal tract (264.87 ± 21.18µg equiv/g).	**X**
	At 78 hours post-dose levels of radioactivity in females had fallen in all tissues except in thyroids where levels seemed to be slightly increased (23.06 8.32µg equiv/g). Highest mean levels of radioactivity were in fat (134.88 ± 25.03µg equiv/g), gastrointestinal tract (56.46 ± 14.35µg equiv/g), adrenals (28.75 ± 3.54µg equiv/g), ovaries (20.16 ± 4.14µg equiv/g), pancreas (19.70 ± 5.15µg equiv/g), liver (19.55 ± 1.02µg equiv/g) and skin (18.74 ± 7.46µg equiv/g). As in male rats, by the last time point (168 hours post-dose), levels of radioactivity in the tissues had continued to fall. Highest mean levels were found in fat (38.54 ± 15.13µg equiv/g), adrenals (13.54 ± 4.63µg equiv/g) , thyroids (12.85 ± 5.73µg equiv/g) and ovaries (9.85 ± 4.44 µg equiv/g).	**X**
	Following administration of [^{14}C]-fipronil at 4 mg/kg bodyweight to male and female rats the distribution of radioactivity was similar to that obtained at the higher dose level. At 0.75 hour post-dose the highest mean levels of radioactivity in male rats, were present in stomach plus contents and in gastrointestinal tract plus contents (146.67 ± 141.56 µg equiv/g and 45.62 ± 24.46µg equiv/g)) in liver (9.17 ± 1.06µg equiv/g), adrenals (9.01 ± 0.68µg equiv/g), pancreas (5.17 ± 1.72µg equiv/g) and thyroids (3.67 ± 0.61µg equiv/g). Mean levels of radioactivity in the remaining tissues were between 3.32 to 0.62 µg equiv/g (kidney and cardiac blood). At 4.8 hours post-dose, mean levels of radioactivity were similar or lower to those detected at 0.75 hour in all tissues other than fat (30.93 ± 11.98µg equiv/g) and skin (5.09 ± 1.17µg equiv/g) where levels increased. Other than fat, highest mean levels were present in gastrointestinal tract (13.36 ± 8.28µg equiv/g) and in adrenals (11.30 ± 1.42µg equiv/g).	**X**

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**	
	By 96 hours post-dose, levels of radioactivity had fallen in all tissues examined except for pancreas where levels seemed to be relatively constant. The highest mean levels detected were in fat (15.83 ± 1.48µg equiv/g), adrenals (8.28 ± 1.75µg equiv/g), pancreas (6.50 ± 0.29µg equiv/g) and skin (4.09 ± 0.09µg equiv/g).	**X**
	At 168 hours post-dose, levels of radioactivity had continued to fall. Highest mean levels were in fat (15.83 ± 1.48µg equiv/g), adrenals (5.24 ± 0.793.38µg equiv/g) pancreas (4.45 ± 0.99µg equiv/g) and skin (3.30 ± 0.42µg equiv/g) with levels in the remaining tissues between 2.36 to 0.22 µg equiv/g (liver and blood). In female rats, the distribution of radioactivity at 0.83 hours was essentially similar to that obtained in male rats killed at 0.75 hours. Highest levels were detected in stomach plus contents and in gastrointestinal tract plus contents (53.13 ± 36.87µg equiv/g and 46.22 ± 3.41µg equiv/g), in fat (12.93 ± 4.82µg equiv/g) , liver (11.71 ± 0.70µg equiv/g), adrenals (10.09 ± 1.93µg equiv/g) and pancreas 6.14 ± 1.06µg equiv/g). Consistent with the females which received the high dose; relatively high levels of radioactivity were detected in gonads (ovaries, 5.88 ± 1.46µg equiv/g). At 6.2 hours post-dose, mean levels of radioactivity in most tissues were similar or lower (i.e. adrenals and ovaries 9.65 ± 0.85µg equiv/g and 5.55 ± 0.82µg equiv/g respectively) than at 0.83 hours post-dose. The exceptions were fat (30.76 ± 02.24 µg equiv/g), uterus (3.87 ± 0.60µg equiv/g) and skin (5.44 ± 0.46µg equiv/g) where levels had increased. Mean levels in all remaining tissues were between 9.37 and 0.56 µg equiv/g (gastrointestinal tract and cardiac blood). No tissues presented levels of radioactivity below the limit of detection. By 94 hours post-dose, levels of radioactivity had fallen in all tissues examined except for ovaries where levels remained relatively constant (5.43 ± 0.80µg equiv/g). The highest mean levels detected were in fat (24.67 ± 3.92µg equiv/g) and in the ovaries.	**X**
	As in male rats, by the last time point (168 hours post-dose), the levels of radioactivity in the tissues had continued to fall. Highest mean levels were in fat (22.48 ± 1.28µg equiv/g), ovaries (4.57 ± 1.94µg equiv/g), adrenals (3.91 ± 0.43µg equiv/g) and in the skin and fur (3.85 ± 0.18µg equiv/g).	**X**

Section A6.2 **Annex Point IIA, VI.6.2**	**Tissue kinetic study in the rat**	
4.3 Conclusion	Following a single oral administration of [^{14}C]-fipronil to male and female rats at the nominal dose levels of 4 and 40 mg/kg bodyweight, the rate of absorption of radioactivity appeared to the independent of sex, but dependent upon dose level with a rapid absorption being observed for the 4 mg/kg 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 a predominance in fatty tissues. The concentrations in the sampled tissues peaked at the blood T_{max} times for both the males and the females with the exception of the tissues involved in the absorption process (stomach and gastrointestinal tract). A difference between the two dose groups was noted between the levels of radioactivity in the stomach and contents. The levels observed in the stomach and contents were among the highest observed levels only at the ½C_{max} absorption sampling time for the 4 mg/kg group whereas levels in the stomach and contents remained high at the /C_{max} sampling time (ca 36 hours post-dose for the 40mg/kg group. The levels observed in the gastrointestinal tract plus contents remained among the highest at all sampling times in both dose groups. The highest levels of absorbed radioactivity were observed in the same tissues for both dose groups and included abdominal fat (consistently containing the highest concentrations of absorbed radioactivity), adrenals, pancreas, thyroids, skin and fur, ovaries, uterus and the liver. The total amount of radioactivity which remained in the tissues at 168 hours post dose was considerably lower for the 40 mg/kg dose group (ca 9% of the administered dose) compared to the 4 mg/kg grop (ca 55% of the administered dose). **The relatively long elimination half-life (ca 183 hours) suggests the presence of a deep compartment such as fat.**	
4.3.1 Reliability	1	
4.3.2 Deficiencies	No	

Table A6.2.9-1 Dosing Regime

Group	Group Type	Dose	Nominal Dose (mg/kg)	Dose Route	No of Rats	Nominal Radioactive dose (µCi/rat)
A	PK	High	40	Oral	5M, 5F	25
B	PK	Low	4	Oral	5M, 5F	25
C	TK	High	40	Oral	12M, 12F	25
D	TK	Low	4	Oral	12M, 12F	25

PK: Blood pharmacokinetics
TK : Tissue Distribution Kinetics

Table A6.2.9-2 Blood pharmacokinetic parameters in rats following as single oral dose of [^{14}C]-fipronil at 4 and 40 mg/kg

Dose Level (mg/kg)	T_{max} (hours)				C_{max} µg equiv/g)				~~t0~~ t1/2 elimination (hours)			
	Males		Females		Males		Females		Males		Females	
	Mean	~~SD~~	Mean	~~SD~~	Mean	SD	Mean	SD	Mean	SD	Mean	SD
40	33.60	~~13.15~~	38.40	~~13.15~~	6.68	1.47	7.58	0.32	135.40	15.80	170.60	27.30
4	4.80	~~1.79~~	6.20	~~2.05~~	0.52	0.06	0.39	0.08	182.50	22.40	245.20	34.80

X X X

Table A6.2.9-3 Mean tissue concentration of radioactivity in rats following a single oral dose of [^{14}C]-fipronil at a nominal dose level of 40 mg/kg

Sample	Concentration of Radioactivity expressed in terms of µg equiv/g							
	Males				Females			
	3h	33.6h	77h	168h	4h	38.4h	78h	168h
Liver	30.96	35.68	16.88	5.76	32.14	32.11	19.55	6.33
Kidney	14.38	17.21	8.84	3.31	15.68	16.04	10.73	3.71
Heart	10.59	12.17	6.79	2.37	11.61	11.86	8.40	2.84
Lungs	14.06	~~7.01~~ 17.01	8.76	3.36	13.71	16.32	10.49	3.38
Brain	8.72	9.68	4.50	1.59	8.75	9.68	5.56	1.97
Spleen	6.35	8.25	4.59	1.28	7.24	7.67	5.21	1.63
Pancreas	31.46	37.69	12.95	6.15	26.11	32.43	19.70	5.59
Muscle	7.55	10.01	4.56	1.50	7.36	8.83	5.85	1.95
Fat	68.63	228.61	114.85	32.05	79.88	201.24	134.88	38.54
Gonads	6.05	9.30	4.24	1.47	20.04	43.97	20.16	9.85
Stomach + contents	380.82	64.41	10.28	0.88	184.86	147.90	8.74	1.31
GIT = contents	421.88	73.26	49.11	4.89	359.97	64.87	56.46	5.57
Bone and marrow	3.39	4.56	3.13	0.96	3.25	4.66	2.58	1.36
Adrenals	34.49	53.93	19.77	15.79	39.15	47.13	28.75	13.54
Cardiac blood	2.09	3.76	2.33	0.65	2.16	5.07	2.93	0.92
Plasma	2.89	5.71	3.34	0.76	2.88	6.23	4.24	1.06
Thyroids	25.14	29.37	16.54	10.45	15.52	15.72	23.06	12.85
Residual carcass	9.87	17.37	8.21	2.70	11.74	14.07	9.13	2.99
Skin and fur	16.95	29.26	15.97	6.36	19.82	29.43	18.74	6.15
Uterus	n/a	n/a	n/a	n/a	17.72	30.49	10.90	7.15

X

GIT Gastrointestinal tract
n/a not applicable

Table A6.2.9-4 Mean tissue concentration of radioactivity in rats following a single oral dose of [^{14}C]-fipronil at a nominal dose level of 4 mg/kg

Sample	Concentration of Radioactivity expressed in terms of µg equiv/g							
	Males				Females			
	0.75h	4.8h	96h	168h	0.83h	6.2h	94h	168h
Liver	9.17	6.84	3.38	2.36	11.71	7.73	3.17	2.89
Kidney	3.32	3.47	2.10	1.48	4.09	3.39	1.75	1.59
Heart	2.28	2.45	1.46	0.88	2.92	2.73	1.40	1.25
Lungs	2.76	3.22	1.70	1.47	3.27	3.10	1.64	1.54
Brain	1.82	2.08	1.13	0.78	2.35	2.31	1.13	0.98
Spleen	1.28	1.45	0.88	0.69	1.67	1.55	0.86	0.78
Pancreas	5.17	6.68	6.50	4.45	6.14	5.27	3.24	2.60
Muscle	1.81	2.98	0.91	0.76	1.79	2.12	0.99	1.27
Fat	10.91	30.93	23.60	15.83	12.93	30.76	24.67	22.48
Gonads	1.44	1.74	0.98	0.96	5.88	5.55	5.43	4.57
Stomach + contents	146.67	0.50	0.33	0.56	53.13	0.73	0.40	0.57
GIT = contents	45.62	13.36	3.25	2.14	46.22	9.37	3.58	2.87
Bone and marrow	0.69	0.84	0.47	0.36	0.93	0.81	0.51	0.41
Adrenals	9.01	11.30	8.28	5.24	10.09	9.65	5.10	3.91
Cardiac blood	0.62	0.62	0.26	0.22	0.68	0.56	0.25	0.23
Plasma	0.81	0.79	0.34	0.27	0.86	0.68	0.33	0.30
Thyroids	3.67	5.08	2.72	2.16	4.15	4.13	2.67	2.86
Residual carcass	1.53	2.24	1.69	1.34	1.91	2.66	1.66	1.61
Skin and fur	1.87	5.09	4.09	3.30	2.40	5.44	3.76	3.85
Uterus	n/a	n/a	n/a	n/a	2.07	3.87	3.34	2.48

GIT Gastrointestinal tract
n/a not applicable

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference : *XXXX*. 17 October 1994 3.1 Test material: ~~*as given in section 2*~~ [^{14}C]-MB 46030 (fipronil) 3.1.2 Specification: ~~*as given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: ~~*stable*~~ The stability was the responsibility of the sponsor 3.2.2 Strain : Sprague-Dawley origin 3.2.6 Number of animals per group: *68* rats in total ~~*(34 male and 34 female)*~~ 10 rats for the blood pharmacokinetics studies (5 females/5 males) and 24 for the tissue distribution kinetics studies (12 females/12 males). 3.3.3 Preparation of test substance: ~~*Suspension in 0.5% carboxymethyl cellulose solution containing 1% Tween 80*~~ Suspension in aqueous methyl cellulose (0.5% w/w) and Tween 80 (0.01% w/v). 3.3.5 Post-exposure period: *168 hours* for the tissue distribution experiments and 336 hours for the pharmacokinetics experiments.
Results and discussion	-
Conclusion	Agree with applicant's version. **Revisions/amendments:** 4.1.1 Treatment: *Groups ~~of 34 rats/sex~~ were either given a single nominal dose of 4 or 40 mg/kg bodyweight [^{14}C]-fipronil by gavage. (Blood pharmacokinetics , Groups A* (10 animals) *and B* (10 animals) *and Tissue Distribution Kinetics Groups C* (24 animals) *and D* (24 animals)*). Fipronil was administered as a suspension in ~~0.5% carboxymethyl cellulose solution containing 1% Tween 80~~* aqueous methyl cellulose (0.5% w/w) and Tween 80 (0.01% w/v). 4.1.2 Sampling: ~~*(Table A6.2.9-2)*~~ (Table A6.2.9-5). Table A6.2.9-5:

Dose Group	Dose Level (mg/kg)	½Tmax (abs) (h)	Tmax (h)	½Tmax (elim) (h)
C males	40	3	33.6	77.0
C females	40	4	38.4	78.0
D males	4	0.75	4.8	96.0
D females	4	0.83	6.2	94.0

	4.1.3 Radioassay: *spectral quench parameter ~~(tie)~~ method*. (tSIE) ~~*4.1 Results*~~: 4.2 Results 4.2 Results: *but had fallen in the gastrointestinal tract ~~(264.87 ± 21.18µg equiv/g).~~* (64.87±21.18 µg equiv./g). *At 78 hours post-dose levels of radioactivity in females had fallen in all tissues except in thyroids where levels seemed to be slightly increased (23.06 ± 8.32µg equiv/g).* *As in male rats, by the last time point (168 hours post-dose), levels of radioactivity in the tissues* in females *had continued to fall.* *At 0.75 hour post-dose the highest mean levels of radioactivity in male rats, were present in stomach plus contents and in gastrointestinal tract plus contents (146.67 ± 141.56 µg equiv/g and 45.62 ± 24.46µg equiv/g)),* fat (10.91± 3.59 µg equiv/g), *in liver (9.17 ± 1.06µg equiv/g), adrenals (9.01 ± 0.68µg equiv/g), pancreas (5.17 ± 1.72µg equiv/g) and thyroids (3.67 ± 0.61µg equiv/g).* *The highest mean levels detected were in fat ~~(15.83 ± 1.48µg equiv/g)~~* (23.60 ± 1.90 µg equiv/g), *adrenals (8.28 ± 1.75µg equiv/g), pancreas (6.50 ± 0.29µg equiv/g) and skin (4.09 ± 0.09µg equiv/g).* *At 168 hours post-dose, levels of radioactivity had continued to fall. Highest mean levels were in fat (15.83 ± 1.48µg equiv/g), adrenals (5.24 ± 0.79~~3.38~~µg equiv/g)* *As in male rats, by the last time point (168 hours post-dose), levels of radioactivity in the tissues* in females *had continued to fall.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3 **Annex Point IIA, VI.6.3**	**Short-term repeated dose toxicity (28 days)**

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.3.1/01** XXXX. M&B 46,030 Toxicity to rats by dietary administration for 4 weeks. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not stated but compliant with EU 92/69/EEC (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes	
		3. MATERIALS AND METHODS	
3.1	**Test material**	Fipronil (M&B 46030)	
3.1.1	Lot/Batch number	IGB 464	
3.1.2	Specification	Deviating from specification given in section 2 as follows: the purity is lower than the minimum in the current specification	
3.1.2.1	Description	A green solid	
3.1.2.2	Purity	93%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley CD	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: approx. 35 days old Weight: 187 – 234 g (males) and 156 – 193 g (females)	
3.2.6	Number of animals per group	10 (5 males and 5 females)	
3.2.7	Control animals	Yes	**X**
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	28 days	
3.3.2	Frequency of exposure	Continuous	
3.3.3	Post exposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	in food	
3.3.4.2	Concentration	Food: 25, 50, 100, 200 & 400 ppm food consumption per day : ad libitum	
3.3.4.3	Vehicle	None	

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

3.3.4.4 Concentration in vehicle	n.a.	
3.3.4.5 Total volume applied	n.a.	
3.3.4.6 Controls	plain diet	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: daily	
3.4.1.2 Mortality	Yes: daily	
3.4.2 Body weight	Yes: weekly	
3.4.3 Food consumption	Yes: weekly and/or part weekly	
3.4.4 Water consumption	Yes: week 3	
3.4.5 Opthalmoscopic examination	Yes: after 4 weeks (controls and top dose only)	
3.4.6 Haematology	yes number of animals: all animals time points: end of study Parameters: Packed cell volume, haemoglobin concentration, platelet count, prothrombin time, red cell count	
3.4.7 Clinical chemistry	yes number of animals: all animals time points: end of study Parameters: sodium, potassium, glucose, total cholesterol, blood urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, globulin , calcium, inorganic phosphorus, chloride, glucose	
3.4.8 Urinalysis	yes number of animals: all animals time points: end of study Parameters: volume, specific gravity, pH, protein, glucose, blood, ketones, bile pigments, urobilinogen	**x**
3.5 Sacrifice and pathology		
3.5.1 Organ weights	yes organs: adrenals, brain, heart, liver, kidneys, adrenals, testes, uterus, ovaries, pituitary thyroid, spleen,	
3.5.2 Gross and histopathology	yes all dose groups organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes peripheral nerve, bone marrow, skin, eyes	
3.5.3 Other examinations		
3.5.4 Statistics	All statistical analyses were carried out separately for males and females. For all parameters, the analyses were carried out using the individual animal as the basic experimental unit. Data relating to food or water consumption were not analysed, as these data were measured on a; cage basis and there was only 1 cage/sex/group.	

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

		The following sequence of statistical tests were used for bodyweight, organ weight and clinical pathology data: 1) If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of animals with values different from the mode was analysed by appropriate methods otherwise: 2) Bartlett's test was applied to test for heterogeneity of variance between treatments. Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was tried to see if a more stable variance structure could have been obtained. 3) If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. If significant heterogeneity of variance was present, and could not be removed by a transformation, the Kruskal-Wallis analysis of ranks was used.	x
3.6	**Further remarks**		
		4. RESULTS AND DISCUSSION	
4.1	**Observations**		
4.1.1	Clinical signs	No effects	
4.1.2	Mortality	One mortality at the highest dose rate (No clinical signs indicative of a reaction to treatment recorded during life and no macroscopic or microscopic finding indicative of the cause of death)	
4.2	**Body weight gain**	(See Table A6.3.1-1) On Day 5 of treatment there was a statistically significant reduction in bodyweight gain at 100 ppm and a dose dependent bodyweight loss at 200 and 400 ppm.	x
4.3	**Food consumption and test compound intake**	Food consumption (see Table A6.3.1-2): On Day 5 of treatment there was a dose dependent reduction in food intake of both sexes at 100, 200 or 400 ppm. Subsequent consumption improved and was similar to that of the controls after one week in females and three weeks in males. Food conversion ratio: Food conversion ratios were dose dependently reduced in Week 1 at 100, 200 and 400 ppm. Test compound intake (see also Table A6.3.1-3): 0– 25– 50– 100– 200– 400 ppm, correspond to mean intake: Males: 0–3.4–6.9–12.6–24.5–45.3 mg/kg bw/d Females: 0–3.5–6.7–12.9–24.9–54.9 mg/kg bw/d	x
4.4	**Ophthalmoscopic examination**	No effects	
4.5	**Blood analysis**		
4.5.1	Haematology	Mean platelet counts were marginally higher than controls in both sexes at 200 and 400 ppm.	x

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

4.5.2	Clinical chemistry	(See Table A6.3.1-4) Group mean total protein and globulin levels were increased in treated animal groups compared with controls but there was no dose-response relationship. In addition, at 400 ppm, there was a marginal reduction in albumin levels in females alone and a small increase in calcium levels in males. Group mean cholesterol levels were increased in both sexes treated at 400 ppm and in females alone given 25 to 200 ppm.	x
4.5.3	Urinalysis	No effects	
4.6	**Sacrifice and pathology**		
4.6.1	Organ weights	(See Table A6.3.1-5) Group mean liver weights relative to terminal bodyweight were increased in all treated males and females although values for males fed 100 ppm were not statistically significant. In addition, there were marginally higher thyroid weights in all groups of treated females. A similar response was seen in males fed 50 or 100 ppm but not at 200 or 400 ppm.	x
4.6.2	Gross and histopathology	Macroscopic pathology (See Table A6.3.1-6): Enlargement of the liver was noted in a number of treated animals, especially at 200 and 400 ppm. Microscopic pathology (see Table A6.3.1-7): Thyroid follicular hypertrophy was found in both sexes of all treated groups. Whilst its severity was generally minimal, moderate hypertrophy occurred in a proportion of males and females given 200 or 400 ppm. A dose-related increase in generalised hepatocyte enlargement was also seen at 200 and 400 ppm an in males alone at 100 ppm.	x
4.7	**Other**		
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of five male and five female Sprague Dawley CD rats were given dietary concentrations of 0, 25, 50, 100, 200 or 400 ppm of Fipronil for four weeks. The test diets were analysed to confirm the homogeneity of mixing and stability of the test material in the diet. Samples of the lowest and highest dietary concentrations from the first and last weeks of treatment were analysed to confirm the accuracy of dose preparation. The rats were approximately 35 days old and weighed 187-234 g (males) and 156-193 g (females) at the start of treatment. They were housed by sex in groups of five and were acclimatised for 7 days before treatment started. Animals were observed daily for clinical signs and mortality. Ophthalmoscopy was conducted on all animals before treatment started and on all control and highest dose level rats in Week 4. Individual bodyweights were recorded at the start of treatment, on Days 5 and 6 of Week 1 and once weekly thereafter. Cage group food consumption was measured on a weekly or part-weekly basis whilst cage group water consumption was determined during Week 3. Clinical chemistry, hematology and urinalysis were performed in Week 4 following overnight deprivation of food and, for those rats sampled for urinalysis, overnight removal of water. All animals were	

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

		necropsied, macroscopic abnormalities of contents of the cranium (brain, pituitary and cranial nerves), thoracic and abdominal cavities were recorded. The weights of selected organs were also noted and a comprehensive range of tissues preserved. A range of tissues from the control and high dose groups was examined microscopically together with abnormalities from all animals. The liver and thyroid from the intermediate dose groups were also examined.	
5.2	**Results and discussion**	Mortality One female from the high dose (400 ppm) died during Week 1 of treatment. No clinical signs were seen and there was no macroscopic or microscopic finding indicative of the cause of death. Since this animal was from the highest dose level, death may have been attributed to treatment with Fipronil. Clinical signs There were no treatment-related clinical signs. Bodyweight (see Table A6.3.1-1): On Day 5 of treatment there was a statistically significant reduction in bodyweight gain at 100 ppm and a dose dependent bodyweight loss at 200 and 400 ppm. These findings were considered to reflect the initial reduction in food consumption in these groups. Subsequent bodyweight gain in these rats was essentially similar to that of the controls. Bodyweight gain at 25 or 50 ppm was unaffected by treatment. Food consumption (see Table A6.3.1-2) On Day 5 of treatment there was a dose dependent reduction in food intake of both sexes at 100, 200 or 400 ppm. Subsequently consumption improved and was similar to that of the controls after one week in females and three weeks in males. Food conversion ratios were dose dependently reduced in Week 1 at 100, 200 and 400 ppm. Achieved test material intake (See Table A6.3.1-3) Water consumption There was no treatment-related effect on water intake. Ophthalmoscopy No treatment-related lesions were found. Hematology Mean platelet counts were marginally higher than controls in both sexes at 200 and 400 ppm. Blood chemistry (see Table A6.3.1-4) Group mean total protein and globulin levels were increased in treated animal groups compared with controls but there was no dose-response relationship. In addition, at 400 ppm, there was a marginal reduction in albumin levels in females alone and a small increase in calcium levels in males. Group mean cholesterol levels were increased in both sexes treated at 400 ppm and in females alone given 25 to 200 ppm of Fipronil. Urinalysis There were no treatment-related changes.	**x** **x** **x** **x**

Section A6.3.1 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (oral)**

		Organ weights (see Table A6.3.1-5) Group mean liver weights relative to terminal bodyweight were increased in all treated males and females although values for males fed 100 ppm were not statistically significant. In addition, there were marginally higher thyroid weights in all groups of treated females. A similar response was seen in males fed 50 or 100 ppm, but not at 200 or 400 ppm. No other treatment-related findings were found.	**x**
		Macroscopic pathology (See Table A6.3.1-6) Enlargement of the liver was noted in a number of treated animals, especially at 200 and 400 ppm.	**x**
		Microscopic pathology (see Table A6.3.1-7) Thyroid follicular hypertrophy was found in both sexes of all treated groups. Whilst its severity was generally minimal, moderate hypertrophy occurred in a proportion of males and females given 200 or 400 ppm. A dose-related increase in generalised hepatocyte enlargement was also seen at 200 and 400 ppm and in males alone at 100 ppm.	**x**
5.3	**Conclusion**	Dietary administration of 50 to 400 ppm of Fipronil to rats for four weeks resulted in generalised hepatocyte enlargement at 100 ppm and above in the liver and follicular hypertrophy thyroid at all dose levels. Other liver responses included mild alterations in some clinical chemistry parameters, increased liver weight and macroscopic liver enlargement at 25 ppm and/or above. Marginally higher thyroid weights occurred in all treated animal groups.	**x**
5.3.1	LO(A)EL	25 ppm (3.4 mg/kg bw/d in males and 3.5 mg/kg bw/d in females)	
5.3.2	NO(A)EL	Less than 25 ppm i.e. <3.4 mg/kg/day in males and 3.5 mg/kg/day	
5.3.3	Other		
5.3.4	Reliability	2	
5.3.5	Deficiencies	Yes: The material used in this study had a purity of 93%. Since the study was conducted the specification for technical fipronil has been modified such that the minimum purity is 95%. This slight difference in purity is unlikely to have a significant impact on the results of the study and would not justify the use of further animals to undertake a further study	

Table ~~A6.3.1-1~~ A6.3.1.1-1 Group Mean bodyweight change (g)

X

Weeks of treatment	**Dose level (ppm)**											
	0	**25**	**50**	**100**	**200**	**400**	**0**	**25**	**50**	**100**	**200**	**400**
	Males						**Females**					
0 – 0.5	34	32	31	15**	-3**	-16**	15	13	15	3**	-4**	-11**
0.5 – 4	140	146	148	148	144	150	66	58	62	60	65	70

** p≤0.01

Table ~~A6.3.1-2~~ A6.3.1.1-2 Group mean food consumption (g/rat/week) X

Week of treatment	Dose level ppm											
	males						females					
	0	25	50	100	200	400	0	25	50	100	200	400
0.1 – 0.5	116	119	116	93	78	53	87	81	83	68	56	43
0.1 – 0.6	146	150	146	121	108	83	112	103	105	88	82	87
0.1 – 1	203	209	205	178	161	128	153	142	145	128	123	143
2	206	216	214	202	193	182	157	174	153	151	143	173
3	213	216	215	208	202	203	160	152	152	146	152	173
4	178	180	187	166	172	169	154	138	138	137	140	157

Table ~~A6.3.1-3~~ A6.3.1.1-3 Group mean material intake (mg/kg bw/d) X

Week	Dose level (ppm)									
	Males					Females				
	25	50	100	200	400	25	50	100	200	400
1 – 4	3.4	6.9	12.6	24.5	45.3	3.5	6.7	12.9	24.9	54.9

Table ~~A6.3.1-4~~ A6.3.1.1-4 Group mean clinical chemistry parameters X

Parameter	Sex	Dose level ppm					
		0	25	50	100	200	400
Total protein (g/dl)	Males	6.5	7.0**	7.0**	7.0**	6.9**	7.0**
	Females	6.3	6.6**	7.0**	6.9**	7.1**	6.9**
Globulin (g/dl)	Males	3.2	3.6**	3.6**	3.6**	3.6**	3.7**
	Females	2.9	3.2*	3.6**	3.6**	3.9**	3.8**
Albumin (g/dl)	Males	3.3	3.4	3.3	3.3	3.3	3.2
	Females	3.3	3.4	3.4	3.3	3.2	3.2*
Calcium (mEq/l)	Males	5.5	5.6	5.6	5.6	5.6	5.7*
	Females	5.5	5.5	5.6	5.6	5.6	5.6
Cholesterol (mg/dl)	Males	85	83	93	86	81	110*
	Females	61	84**	106**	105**	115**	139**

* $p \leq 0.05$ ** $p \leq 0.01$

Table ~~A6.3.1-5~~ A6.3.1.1-5 Group mean liver and thyroid weights X

Organ	Dose level ppm											
	males						females					
	0	25	50	100	200	400	0	25	50	100	200	400
Terminal body weight (g)	372	378	379	363	345	337	252	240	245	230	232	230
Liver (g)	18.6	20.4 (110)	21.6 (116)	21.2 (114)	21.7* (117)	25.6** (138)	11.6	12.3 (106)	14.3 (123)	13.6 (117)	15.6 (134)	16.6 (143)

Rel. liver weight %[a]	–	–	–	–	–	–	10.5	12.1* (115)	13.8** (131)	14.3** (136)	16.1** (153)	17.3** (165)
Relative thyroid (mg)	20.4	20.5 (100)	24.3 (119)	24 (118)	20.3 (100)	21.7 (106)	16.6	19.4 (117)	19.5 (117)	18.4 (111)	19.7 (119)	~~118.6~~ 18.6 (112)

Statistical analysis: * = p≤0.05; ** = p≤0.01
% of control in parenthesis
[a] Liver weight adjusted for terminal body weight
- no data

X

Table ~~A6.3.1-6~~ A6.3.1.1-6 Results Group incidence of liver enlargement

X

Finding	Dose level ppm											
	males						females					
	0	25	50	100	200	400	0	25	50	100	200	400
Liver enlargement	0/5	0/5	0/5	1/5	0/5	5/5	0/5	0/5	1/5	0/5	2/5	3/5

Table ~~A6.3.1-7~~ A6.3.1.1-7 Group incidence of thyroid and liver histpathological findings

X

Finding	Dose level ppm											
	males						females					
	0	25	50	100	200	400	0	25	50	100	200	400
Thyroid												
-Follicular hypertrophy												
minimal	0/5	5/5	4/5	5/5	3/5	2/5	0/5	3/5	4/5	3/5	5/5	4/5
moderate	0/5	0/5	0/5	0/5	2/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5
total	**0/5**	**5/5**	**4/5**	**5/5**	**5/5**	**5/5**	**0/5**	**3/5**	**4/5**	**3/5**	**5/5**	**4/5**
Liver												
-Hepatocyte enlargement												
minimal	0/5	0/5	0/5	1/5	3/5	5/5	0/5	0/5	0/5	0/5	2/5	4/5
total	**0/5**	**0/5**	**0/5**	**1/5**	**3/5**	**5/5**	**0/5**	**0/5**	**0/5**	**0/5**	**2/5**	**4/5**

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with the applicant's version **Revisions/amendments:** 3.2.7 Control animals: 5 males and 5 females 3.4.4 Water consumption: over daily periods during *week 3* 3.4.5 Opthalmoscopic examination: ~~*after 4 weeks*~~ during week 4 3.4.8 Urinalysis: Microscopy: parameters: epithelial cells, polymorphonuclear leucocytes, mononuclear leucocytes, erythrocytes, organisms, renal tubule casts, sperm and other abnormal constituents. 3.5.4 Statistics: 4) Analyses of variance was followed by Student's 't' test and Williams' test for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the 't' test and Williams' test (Shirley's test).
Results and discussion	Agree with the applicant's version **Revisions/amendments:** 4.2 Bodyweight gain/5.2 Results and discussion: ~~*(See Table A6.3.1-1)*~~ (See Table A6.3.1.1-1) 4.3 Food consumption and test compound intake/5.2 Results and discussion: ~~*(See Table A6.3.1-2)*~~ (See Table A6.3.1.1-2) ~~*(See Table A6.3.1-3)*~~ (See Table A6.3.1.1-3) 4.5.1 Haematology: *Mean platelet counts were marginally higher than controls in both sexes at 200 and 400 ppm.* However, the difference from the control value for the female subgroups did not achieve a level of statistical significance (0.05<P). 4.5.2 Clinical chemistry/5.2 Results and discussion: ~~*(See Table A6.3.1-4)*~~ (See Table A6.3.1.1-4) 4.6.1 Organ weights/5.2 Results and discussion: ~~*(See Table A6.3.1-5)*~~ (See Table A6.3.1.1-5) *In addition, there were marginally higher thyroid weights in all groups of treated females* ~~*A similar response was seen in males fed 50 or 100 ppm but not at 200 or 400 ppm.*~~ and males only treated with 100 or 150 ppm . However, the differences from the control values did not attain a level of statistical significance. Males treated with 400 or 200 ppm were not similarly affected. 4.6.2 Gross and histopathology/5.2 Results and discussion: ~~*(See Table A6.3.1-6)*~~ (See Table A6.3.1.1-6) ~~*(See Table A6.3.1-7)*~~ (See Table A6.3.1.1-7) *Whilst its severity was generally minimal, moderate hypertrophy occurred in a proportion of males* ~~*and females*~~ *given 200 or 400 ppm.*
Conclusion	Agree with the applicant's version

	Revisions/amendments: 5.3 Conclusion: *Marginally higher thyroid weights occurred in all treated ~~animal~~* female *groups*. A similar response was seen in males fed 50 or 100 ppm.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	

	1. REFERENCE	**Official use only**
1.1 Reference	**A6.3.2/01** XXXX M&B 46030: Twenty-one day repeated cutaneous dose toxicity study in New Zealand White rabbits #2. 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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes USEPA (=EPA) 82-2 (1984) Also compliant with OECD 410 (1981)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	x
3.1.1 Lot/Batch number	78/GC/90	
3.1.2 Specification	As given in Section 2	x
3.1.2.1 Description	White powder	
3.1.2.2 Purity	96.7%	
3.1.2.3 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Rabbit	
3.2.2 Strain	New Zealand White	
3.2.3 Source	XXXX	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Age: approx. 18 and 19 weeks male and female, respectively Weight: 3.0 – 3.6 kg (males) and 3.1– 3.8 kg (females)	
3.2.6 Number of animals per group	12 – 6 males and 6 females	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	Dermal	
3.3.1 Duration of treatment	21 days	
3.3.2 Frequency of exposure	daily	x
3.3.3 Postexposure period	none	
3.3.4 **Dermal**	Dose levels: 0, 0.5, 1, 5 or 10 mg/kg bw/d of Fipronil	
3.3.4.1 Area covered	4 x 4 inch = approx. 10 x 10 cm	
3.3.4.2 Occlusion	occlusive	
3.3.4.3 Vehicle	0.5% aqueous carboxymethyl cellulose	
3.3.4.4 Concentration in vehicle	0.5, 1, 5 and 10 mg/ml	

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	
3.3.4.5 Total volume applied	1 ml/kg/day	
3.3.4.6 Duration of exposure	6 hours per day	
3.3.4.7 Removal of test substance	Application site was wiped with a dampened cloth	
3.3.4.8 Controls	0.5% aqueous carboxymethyl cellulose	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: daily	x
3.4.1.2 Mortality	Yes: daily	
3.4.2 Body weight	Yes: weekly	x
3.4.3 Food consumption	Yes: every other day	
3.4.4 Water consumption	No	
3.4.5 Opthalmoscopic examination	No	
3.4.6 Haematology	Yes Number of animals: all animals Time points: End of study Parameters Heanatocrit, haemoglobin, erythrocyte count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, total leukocyte count, differential leukocyte count, platelet count	
3.4.7 Clinical chemistry	Yes Number of animals: all animals Time points: End of study Parameters: glucose (fasting), urea nitrogen, creatinine, total protein, albumin, globulin (calculated) total bilirubin, calcium, phosphorus, sodium potassium, chloride, aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactate dehydrogenase, gamma-glutamyl transferase, sorbitol dehydrogenase, alkaline phosphatase	
3.4.8 Urinalysis	No	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes all dose groups organs: Liver, kidneys, adrenals, testes, and ovaries	
3.5.2 Gross and histopathology	Yes <u>Macroscopic pathology:</u> full internal and external examination (all animals) Microscopic pathology: treated and untreated skin, liver, kidneys (control and high dose animals);gross lesions (all animals)	
3.5.3 Other examinations		

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	
3.5.4 Statistics	The data for quantitive continuous variables were intercompared for the 4 treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used only when only one group was compared to the control group or the F value from the ANOVA was significant. When Levene's test indicated similar variance, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary , by a separate variance t-test for pairwise comparisons Non-parametric data were statistically evaluated using the Kruskal-Wallis test followed by the Mann-Whitney U-test. Incidence data were compared using Fisher's Exact Test. For all statistical tests a probability value of < 0.05 (two-tailed) was used as the critical level of significance.	
3.6 Further remarks	**4. RESULTS AND DISCUSSION**	
4.1 Observations		
4.1.1 Clinical signs	At 10 mg/kg bw/d, one male and one female exhibited a period of extreme hyperactivity near the end of the study. Both animals recovered. Since spasms and delayed convulsions were observed in a previous acute dermal toxicity study with Fipronil, these findings were considered to be treatment-related. No erythema or oedema was observed in any animals.	
4.1.2 Mortality	There were no deaths	
4.2 Body weight gain	At 10 mg/kg bw/d, bodyweight was reduced in males by 4 and 7% on Days 15 and 21, respectively. Although bodyweight gain was decreased at all time points, statistical significance was only achieved for the overall value (Days 1 to 21). No treatment-related effects were seen at lower dose levels.	x
4.3 Food consumption	(See Table A6.3.2-2) At 10 mg/kg bw/d, food consumption was reduced in males and females by 44 and 22%, respectively, between Days 15 and 21 although only the value for males was statistically significant. The decrease in consumption between Days 8 and 15 was principally due to two rabbits and was not significant. There were no other treatment-related effects. The statistically significant increase in food consumption of the 5 mg/kg bw/d group during the Day 8 to 15 interval was not considered to be related to treatment due to the lack of a dose-response relationship.	x
4.4 Ophthalmoscopic examination	n.a.	
4.5 Blood analysis		
4.5.1 Haematology	No effects	
4.5.2 Clinical chemistry	No effects	
4.5.3 Urinalysis	n.a	

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	In both male and female rabbits, there were no effects on the absolute or relative organ weights in an dose group. The slight decrease in absolute body weight observed in male rabbits from the high dose group on Day 21 was also reflected in the final body weight but this change was not statistically significant. Statistically significant increases in the absolute and relative weight of the adrenal glands in the 0, 5 and 1 mg/kg/day groups of male rabbits were not considered to be related to treatment due to the lack of a dose-response relationship.	
4.6.2 Gross and histopathology	No effects	
4.7 Other		
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Groups of six male and six female New Zealand White rabbits were given a 6-hour dermal application for 5 days each week for 3 weeks (15 doses in total) of 0, 0.5, 1, 5 or 10 mg/kg bw/d of Fipronil, suspended in 0.5% aqueous carboxymethyl cellulose. Controls received the vehicle alone. Doses were applied at a volume of 1.0 ml/kg bw/d to the clipped dorsal skin under a gauze pad and the rabbit was then wrapped in a Lycra®/Spandex® jacket lined with polyvinyl sheeting and secured with Velcro® strips. The rabbits were about 18 (males) and 19 (females) weeks old at the start of treatment and weighed between 3.0 to 3.6 kg (males) and 3.1 to 3.8 kg (females). They were housed individually and were acclimatised for about 2 weeks. Stability was assessed following 7 and 14 days storage at room temperature of 0.5 and 10 mg/ml of Fipronil in 0.5% carboxymethylcellulose. homogeneity of mixing was assessed using the dose preparations from Day 1. The achieved concentrations at all dose levels were evaluated in each weekly dose preparation. Animals were observed twice daily for mortality. Detailed clinical observations, including skin irritation scores (using a modified Draize scoring system) were conducted daily. Individual bodyweights were recorded prior to the start of treatment (Day 1), on Days 8, 15 and 21 and at necropsy. Food consumption was measured for each animal approximately every other day. In addition, blood samples for hematology and clinical chemistry were taken from all animals prior to necropsy, following about 24 hours of fasting. All animals were necropsied and examined externally and internally. Selected organs were weighed and a comprehensive range of tissues preserved. Treated and untreated skin, liver, kidneys and all gross lesions from all control and high dose rabbits were examined microscopically.	

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	

5.2	**Results and discussion**	Analysis of dose preparations Acceptable stability was demonstrated in the dose preparations for Day 1 following 7 and 14 days of storage at room temperature. The mean analysed concentrations of Fipronil were 86.7 to 96.9% of nominal at 0.5 mg/ml and 91.1 to 95.1% at 10 mg/ml. Furthermore, satisfactory homogeneity was demonstrated by mean concentrations of 89.0 to 91.8% of nominal and coefficients of variation between 0.6 and 3.1% at 0.5, 1, 5 and 10 mg/kg. Analysis of the dose preparations prepared at weekly intervals showed acceptable levels of Fipronil (88.4 to 96.0% of nominal). Mortality There were no deaths. Clinical signs At 10 mg/kg bw/d, one male and one female exhibited a period of extreme hyperactivity near the end of the study. Both animals recovered. Since spasms and delayed convulsions were observed in a previous acute dermal toxicity study with Fipronil, these findings were considered to be treatment-related. Dermal irritation No erythema or oedema was observed in any animals. Bodyweight At 10 mg/kg bw/d, bodyweight was reduced in males by 4 and 7% on Days 15 and 21, respectively. Although bodyweight gain was decreased at all time points, statistical significance was only achieved for the overall value (Days 1 to 21). No treatment-related effects were seen at lower dose levels. Food consumption At 10 mg/kg bw/d, food consumption was reduced in males and females by 44 and 22%, respectively, between Days 15 and 21 although only the value for males was statistically significant. The decrease in consumption between Days 8 and 15 was principally due to two rabbits and was not significant. There were no other treatment-related effects. Haematology and clinical chemistry There were no treatment-related effects. Organ weights No treatment-related effects on absolute or relative organ weights were found. Macroscopic pathology There were no treatment-related findings. Histopathology No microscopic findings were considered to be related to treatment.	**x**
5.3	**Conclusion**	Dermal application of up to 10 mg/kg bw/d to New Zealand White rabbits over 21 days caused an episode of extreme hyperactivity in one male and one female near the end of the study. Bodyweight gain in both sexes and food consumption, particularly in males were reduced. The No Observed Effect Level (NOEL) was 5 mg/kg bw/d.	
5.3.1	LO(A)EL	10 mg/kg bw/d	
5.3.2	NO(A)EL	5 mg/kg bw/d	

Section A6.3.2 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (dermal)**	

5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	

Table ~~A6.3.2-1~~ A6.3.2.1-1 Group mean bodyweight and bodyweight change (g) X

Day	Dose level (mg/kg bw/d)									
	Males					Females				
	0	**0.5**	**1**	**5**	**10**	**0**	**0.5**	**1**	**5**	**10**
	Bodyweight									
1	3313.7	3370.8	3328.1	3337.3	3323.1	3391.8	3410.7	~~3422.8~~ 3409.0	3422.8	3451.0
8	3382.4	3461.7	3419.1	3489.1	3352.0	3490.8	3512.7	~~3539.8~~ 3527.6	3539.8	3524.9
15	3414.5	3546.8	3491.4	3558.5	3287.2	3584.1	3606.5	~~3635.5~~ 3652.1	3635.5	3548.2
21	3539.0	3640.4	3585.2	3648.9	3275.7*	3644.0	3728.6	3768.7	3769.4	3575.7
Days	Bodyweight change									
1-8	68.7	90.9	91.0	151.8*	28.9	99.0	102.0	118.5	117.1	73.9
1-15	100.7	176.0	163.4	221.2	-35.9	156.3	195.8	243.0	212.7	97.2
1-21	225.2	269.6	257.1	311.6	-47.4**	252.2	317.9	359.7	346.6	124.8

X
X
X

Statistical analysis: *= p≤0.05; **= p≤0.01

Table ~~A6.3.2-2~~ A6.3.2.1-2 Group mean food consumption (g/animal/week) X

Days	Dose level (mg/kg bw/d)									
	Males					Females				
	0	0.5	1	5	10	0	0.5	1	5	10
1-8	193.1	209.8	213.7	2225.	186.1	212.4	218.4	226.2	233.9	223.0
8-15	176.9	202.2	197.1	200.8	146.7	190.9	224.7	233.8*	229.5	175.4
15-~~12~~ 21	206.7	200.5	214.8	204.7	115.8**	202.0	227.7	243.0	237.0	158.6

X

*p≤0.05 **p≤0.01

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	February 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.3.2 Frequency of exposure: ~~*daily*~~. Animals were occluded for 6 hours/day for 5 consecutive days (Monday through Friday for males and Tuesday through Saturday for females). 3.4.1.1 Clinical signs: including skin irritation (modified Draize scoring system) 3.4.2 Body weight: Days 1, 8, 15, 21 and immediately preceding sacrifice.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.2 Body weight gain: (See Table A6.3.2.1-1) Approximately half of the animals lost absolute body weight during the day 1 to 15 and day 1 to 21 intervals. The statistically significant increase in body weight gain of the 5.0 mg/kg/day dose group during the day 1 to 8 measurement interval was not considered to be related to treatment due to the lack of a dose-response relationship. In the high dose group of female, mean absolute body weight gains were decreased 25, 38 and 51 % at the day 1 to 8, 1 to 15 and 1 to 21 measurement intervals, respectively, but these changes were not statistically significant. 4.3 Food consumption: ~~*(See Table A6.3.2-2)*~~ (See Table A6.3.2.1-2) 5.2 Results and discussion: *Analysis of dose preparations* ~~*Acceptable stability was demonstrated in the dose preparations for Day 1 following 7 and 14 days of storage at room temperature.*~~ Dosing suspensions were analyzed for concentration of Fipronil directly after preparation (day 0) and following 7 and 14 days of storage at room temperatude. *The mean analysed concentrations of Fipronil were 86.7 to 96.9% of nominal at 0.5 mg/ml and 91.1 to 95.1%* of nominal *at 10 mg/ml.* Results of this stability study indicated that M&B 46030 in 0.5% CMC remained stable at the specified concentrations when stored at room temperature. ~~*Furthermore satisfactory homogeneity was demonstrated by mean concentrations of 89.0 to 91.8% of nominal and coefficients of variation between 0.6 and 3.1% at 0.5, 1, 5 and 10 mg/kg*~~ Homogeneity was evaluated to ensure the M&B 46030 was uniformly distributed throughout each suspension. Duplicate samples were analyzed from 3 separate regions (top, middle, bottom) of the flask for the 0.5 and 10 mg/ml suspensions. The mean concentrations were 90.1, 89.0, 91.7 and 91.8 % of nominal and coefficients of variation 2.8, 0.6, 3.1 and 1.4 % at 0.5, 1, 5 and 10 mg/ml, respectively. These results indicated that the distribution of fipronil in 0.5% CMC was uniform.

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

Section A6.3.3 **Annex Point IIA, VI.6.3**	**Repeated dose toxicity (inhalation)**	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	(1) The vapour pressure of Fipronil (IIA, 3.2) was determined experimentally under GLP to a value below 3.7 x 10^{-9} hPa (25°C). Therefore, Fipronil is not a volatile substance. (2) In consideration of the intended uses, inhalation is considered to be a negligible route of exposure. In conclusion, the performance of a 28-day inhalation toxicity study in the rat is consequently not considered to be required, predominantly for lack of exposure.	
Undertaking of intended data submission [ ]		

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

3Section A6.4 **Annex Point IIA, VI.6.4**	**Subchronic toxicity**

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A6.4.1/01** XXXX M&B 46030: Toxicity study by dietary administration to CD rats for 13 weeks. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 82-1 (1984). Also compliant with EU 88/302/EEC, B.26 (1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Specific neurological examinations were also conducted	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	Fine white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley CD	
3.2.3	Source	XXXX	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: Approximately 5 – 7 weeks Weight: 154 – 203 g (Males) and 135 – 180 g (females)	
3.2.6	Number of animals per group	20; 10 males and 10 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Continuous	
3.3.3	Post exposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	in food	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
3.3.4.2 Concentration	Food: 1, 5, 30 & 300 ppm food consumption per day : ad libitum	
3.3.4.3 Vehicle	None	
3.3.4.4 Concentration in vehicle	n.a.	
3.3.4.5 Total volume applied	n.a.	
3.3.4.6 Controls	plain diet	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: twice daily	
3.4.1.2 Mortality	Yes: twice daily	
3.4.2 Body weight	Yes: weekly	X
3.4.3 Food consumption	Yes: weekly	
3.4.4 Water consumption	Yes: assessed by visual examination. Quantitative measurements not carried out	
3.4.5 Ophthalmoscopic examination	Yes: after 12 weeks (controls and top dose only)	
3.4.6 Haematology	Yes number of animals: all animals time points: after 12 weeks Parameters: Packed cell volume, haemoglobin concentration, erythrocyte count, Reticulocyte count, Total leucocyte count, differential leucocyte count, platelet count, mean haemoglobin, mean cell volume, mean cell haemoglobin concentration, prothrombin time	
3.4.7 Clinical chemistry	Yes number of animals: all animals time points: after 12 weeks Parameters: sodium, potassium, glucose, blood urea creatinine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, calcium, inorganic phosphorus, chloride, bilirubin	
3.4.8 Urinalysis	Yes number of animals: all animals time points: after 12 weeks Parameters: appearance, volume, specific gravity, pH, protein, glucose, blood, ketones, bile pigments, urobilinogen	X
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes Organs: adrenals, brain, heart, liver, kidneys, lungs with mainstem bronchi, testes, uterus, ovaries, pituitary thyroid with parathyroid, spleen, prostrate, salivary glands, thymus	
3.5.2 Gross and histopathology	Yes all dose groups Organs: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes peripheral nerve, bone marrow, skin, eyes	X
3.5.3 Other examinations		X

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
3.5.4 Statistics	The significance of inter-group differences in bodyweight gain, haematology, blood chemistry and urine data (where appropriate) were assessed by Student's t-test using a pooled within-group error variance. The results of this test are not reported for eosinophil, monocyte, basophil, reticulocyte or large unidentified cell counts for which the data are clearly not normally distributed . For organ weights, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behren's-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. The group distribution of macroscopic and histopathological findings, expressed as incidences, were tested for statistical significance, where considered appropriate, using Fishers exact probability test as a two-tailed test.	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Observations		
4.1.1 Clinical signs	A single incidence of a clonic convulsion seen in Week 9 in one high dose level male fed 300 ppm was considered to be related to treatment. A greater overall incidence of clinical signs was recorded for females receiving 300 ppm than for the controls, particularly in respect of tail encrustation or abrasion. However, a similar change was seen in two control females and therefore this observation could not be confidently attributed to treatment.	
4.1.2 Mortality	none	
4.2 Body weight gain	(See Table A6.4.1.1-1) At 30 ppm, male body weight gain was slightly reduced in Week 1 and, at 300 ppm, body weight gain was markedly reduced during the first week of treatment. However, the overall weight gain of females at this high dose level was only slightly lower than controls and that of males was similar to controls.	X
4.3 Food consumption and compound intake	Food consumption (see Table A6.4.1.1-2): At 300 ppm, food consumption by males was markedly lower than the controls during Weeks 1 and 2 of treatment but females were affected only during Week 1. Intake by males given 30 ppm was also reduced in Week 1. Food conversion was reduced at 300 ppm during the first week of treatment but subsequently conversion by males at this dose level was higher than in controls. Test compound intake (see Table A6.4.1.1-3): The overall achieved dosages for animals receiving 1, 5, 30 or 300 ppm were for males 0.07, 0.3, 1.9 and 20 mg/kg bw/d and for females 0.07, 0.4, 2.3 and 24 mg/kg bw/d, respectively.	X
4.4 Ophthalmoscopic examination	No effects	
4.5 Blood analysis		

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
4.5.1 Haematology	(See Table A6.4.1.1-4) At 300 ppm, low packed cell volume, mean cell volume, mean cell hemoglobin, prothrombin time and high platelet counts were recorded in females. In males, low hemoglobin concentrations were noted. At 30 ppm, low prothrombin times were found in females. All other values were either within the historical control range or were not clearly attributable to treatment.	
4.5.2 Clinical chemistry	(See Table A6.4.1.1-5) After 12 weeks of treatment with 300 ppm of Fipronil, total protein concentrations were higher than in controls. This was associated with lower albumin: globulin ratios and with higher α_1-, α_2- and β-globulins. Similar dose-related responses were seen at 5 and 30 ppm but without any changes to the albumin: globulin ratio. Higher urea concentration was found in all groups of treated males. Aspartate aminotransferase activity was lower in all treated female groups whilst alanine aminotransferase was low at 30 and 300 ppm. Plasma glucose was slightly increased in females at 5 ppm and above.	
4.5.3 Urinalysis	No effects	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	(See Table A6.4.1.1-6) At the 300 ppm highest dose level, absolute and body weight-relative thyroid weights were higher than controls. A similar tendency was found at 30 ppm although statistical significance was only attained for absolute weights for females. Absolute liver weights were significantly increased in the males given 300 ppm and in females fed 5 ppm and above. Body weight-relative weights were increased in both sexes at 30 and 300 ppm. All other inter-group differences were not dose-related or were considered to have arisen by chance.	X
4.6.2 Gross and histopathology	<u>Macropathology</u> There were no treatment related macroscopic changes. Histopathology (see Table A6.4.1.1-7): An increased incidence of thyroid follicular epithelial hypertrophy was observed at 300 ppm. Although the frequency of follicular cell hyperplasia was higher than in controls, this was not statistically significant. In the liver, a low incidence of panacinar fatty vacuolation was also seen in both sexes given 300 ppm. Although staining with Oil red O showed a higher incidence of fat in livers from all groups, including controls, its incidence was significantly higher only in the 300 ppm males where the distribution was more widespread (panacinar compared with centriacinar in controls). No real differences were seen in the livers of females stained with Oil-Red-O.	
4.7 Other	Neurological examination after 12 weeks of treatment for the controls an animals receiving 300 ppm revealed no evidence of any abnormalities for any animal	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Groups of 10 males and 10 female Sprague Dawley CD rats were given dietary concentrations of 0, 1, 5, 30 or 300 ppm of Fipronil for 13 consecutive weeks. Prior to the start of treatment, a trial mix was prepared to assess homogeneity of mixing the test material with the diet at the lowest and highest concentrations. Its stability in this medium after 7 and 14 days of storage at room temperature was also determined at the lowest and highest concentrations. In addition, diet samples from all concentrations fed in Weeks 1 and 13 were analysed for the achieved concentration of Fipronil. At the start of treatment, the rats were 5 to 7 weeks of age and weighed between 154 to 203 g (males) and 135 to 180 g (females). They were acclimatised for 13 days prior to the start of treatment and housed by sex in groups of 5. Animals were observed at least twice daily for mortality and clinical signs. A detailed weekly examination, including a palpation, was also performed. Examination of the eyes was conducted on all animals prior to the start of treatment and on all rats from the control and highest dose groups during Week 12. Individual bodyweights and food consumption were recorded weekly. Blood samples for hematology and clinical chemistry evaluations were taken from all animals during Week 12 whilst urine samples were collected from all rats in Week 13. After 12 weeks of treatment, a neurological examination was carried out on all control and high dose group animals during Week 13. This comprised testing of reflexes, reactions and general observations (cranial reflexes: pupillary, palpebral, startle and general examination of the head for other cranial nerve function; segmental reflexes: flexor withdrawal; postural reactions such as placing and righting reactions and grasping; general observations: behavioural changes, gait abnormalities and tremors or other dyskinesias). All animals were necropsied and examined externally and internally (contents of the cranial, thoracic, abdominal and pelvic cavities) for macroscopic changes. Selected organs were weighed and a comprehensive range of tissues preserved and examined microscopically.	X
5.2 Results and discussion	<u>Analysis of diet preparations</u> Homogeneity of mixing Fipronil with the diet and its stability in this medium following up to 14 days of storage at room temperature was confirmed prior to the start of treatment at the lowest and highest concentrations. Results of samples taken from all dose levels in Weeks 1 and 13 to verify the achieved concentrations gave satisfactory mean values of 93%, 97%, 105% and 96% at 1, 5, 30 and 300 ppm, respectively. Mortality There were no deaths. Clinical signs No treatment-related clinical signs were reported. However, subsequent to the issue of the report of this study, the single incident of clonic convulsion in one 300 ppm high dose male in Week 9 was considered to be treatment-related based on the known pharmacological properties of Fipronil.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
	Neurological examination There was no evidence of any abnormalities. Ophthalmoscopy No treatment-related ophthalmic lesions were found. Bodyweight At 30 ppm, male body weight gain was slightly reduced in Week 1 and, at 300 ppm, body weight gain was markedly reduced during the first week of treatment. However, the overall weight gain of females at this high dose level was only slightly lower than controls and that of males was similar to controls. Food consumption At 300 ppm, food consumption by males was markedly lower than the controls during Weeks 1 and 2 of treatment but females were affected only during Week 1. Intake by males given 30 ppm was also reduced in Week 1. Food conversion was reduced at 300 ppm during the first week of treatment but subsequently conversion by males at this dose level was higher than in controls. <u>Achieved intake of test material</u> The estimated overall mean intakes of Fipronil are shown in the table below. Hematology At 300 ppm, low packed cell volume, mean cell volume, mean cell hemoglobin, prothrombin time and high platelet counts were recorded in females. In males, low hemoglobin concentrations were noted. At 30 ppm, low prothrombin times were found in females. All other values were either within the historical control range or were not clearly attributable to treatment. <u>Clinical chemistry</u> After 12 weeks of treatment with 300 ppm of Fipronil, total protein concentrations were higher than in controls. This was associated with lower albumin: globulin ratios and with higher α_1-, α_{2-} and β-globulins. Similar dose-related responses were seen at 5 and 30 ppm but without any changes to the albumin: globulin ratio. Higher urea concentration was found in all groups of treated males. Aspartate aminotransferase activity was lower in all treated female groups whilst alanine aminotransferase was low at 30 and 300 ppm. Plasma glucose was slightly increased in females at 5 ppm and above. <u>Urinalysis</u> There were no treatment-related findings. <u>Organ weights</u> At the 300 ppm highest dose level, absolute and body weight-relative thyroid weights were higher than controls. A similar tendency was found at 30 ppm although statistical significance was only attained for absolute weights for females. Absolute liver weights were significantly increased in the males given 300 ppm and in females fed 5 ppm and above. Body weight-relative weights were increased in both sexes at 30 and 300 ppm. All other inter-group differences were not dose-related or were considered to have arisen by chance.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
	Macropathology There were no treatment related macroscopic changes. Histopathology An increased incidence of thyroid follicular epithelial hypertrophy was observed at 300 ppm. Although the frequency of follicular cell hyperplasia was higher than in controls, this was not statistically significant. In the liver, a low incidence of panacinar fatty vacuolation was also seen in both sexes given 300 ppm. Although staining with Oil red O showed a higher incidence of fat in livers from all groups, including controls, its incidence was significantly higher only in the 300 ppm males where the distribution was more widespread (panacinar compared with centriacinar in controls). No real differences were seen in the livers of females stained with Oil red O. **Discussion** Subsequent to the issue of this report, based on the known pharmacological properties of Fipronil, the single incidence of a clonic convulsion seen in Week 9 in one high dose level male fed 300 ppm was considered to be related to treatment. During the first week of treatment, reductions in growth, food intake and food conversion were seen at this dose level. Low food intake was also seen in Week 2. Subsequently, animals adapted to treatment, particularly males which exhibited superior weight gain and food conversion ratios. Males fed 30 ppm also showed lower weight gain and food intake during Week 1. The increased weight gain and food intake of females at 5 and 30 ppm might reflect a change in their metabolism (see below). 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. The study report also concluded that the tendency for increases in liver and thyroid weights observed at 30 ppm and changes in serum protein levels were not toxicologically relevant in the absence of histopathological changes. However, given the observed induction of minimal follicular cell hypertrophy at and above 25 ppm in the 28-day oral toxicity study in rats (see section A6.3.1/01) the minimal thyroid weight increases seen at 30 ppm in this 90-day study could be considered to represent an adverse effect.	X

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in rats (90-day study)**	
5.3 Conclusion	At the highest tested dose of 300 ppm, increased fat deposition in male liver was noted and evidence for a minor disturbance of red cell parameters was seen in females. The thyroid follicular epithelial hypertrophy and higher frequency of thyroid follicular hyperplasia at 300 ppm, together with the hepatic changes observed, are indicative of a possible alteration of the hypothalamic-pituitary-thyroid-liver axis. Thus, enhanced clearance of thyroxine by the liver resulting from increased metabolic activity would have lead to increased hypothalamo-pituitary secretion of thyroid stimulating hormone (TSH) which, in turn, resulted in enhanced stimulation of the thyroid gland. Dietary administration of 300 ppm of Fipronil to rats for 13 weeks resulted in an initial reduction in food consumption and associated reduction in body weight gain. Histopathological changes were hepatocyte hypertrophy and hepatocytic fat deposition in the liver and thyroid follicular cell hypertrophy and some thyroid follicular cell hyperplasia. Associated increases in the weights of these organs, together with clinical chemistry changes, were indicative of enhanced liver metabolism in response to administration of a xenobiotic. A tendency towards higher liver and thyroid weights was also observed at 30 ppm.	
5.3.1 LO(A)EL	30 ppm (corresponding to 1.93 mg/kg bw/d in males and 2.28 mg/kg bw/d in females)	
5.3.2 NO(A)EL	5 ppm (corresponding to 0.33 mg/kg bw/d in males and to 0.37 mg/kg bw/d in females and 0.35 mg/kg bw/d for both sexes combined).	
5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	

Table A6.4.1.1-1 Group mean bodyweight change (g)

Week	**Dose level (ppm)**									
	Males					**Females**				
	0	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
	Body weight									
0	182	185	180	181	178	153	155	153	153	155
1	247	248	243	241	211	184	190	188	185	174
2	306	307	301	301	274	207	217	212	213	197
4	398	398	393	396	374	241	252	256	254	233
13	552	571	552	562	546	303	322	327	322	292
	Body weight change									
0-1	65 (100)	64 (98)	63 (97)	60* (92)	33*** (51)	31 (100)	35 (113)	35 (113)	32 (103)	19*** (61)
~~1-13~~ 0-13	370 (100)	386 (104)	372 (101)	381 (103)	368 (99)	150 (100)	167 (111)	173 (115)	168 (112)	136 (91)

% of control in parenthesis
* $p \leq 0.05$ *** $p \leq 0.001$

X

Table A6.4.1.1-2 Group mean food consumption (g)

Week	Dose level (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
1	187	189	183	175	138	142	147	142	147	122
2	193	195	182	183	174	140	142	144	149	144
4	200	194	195	191	193	138	137	~~142~~ 144	~~142~~ 145	146
Total for Weeks 1 - 13	2421 (100)	2438 (101)	2397 (99)	2351 (97)	2340 ~~(197)~~ (97)	1742 (100)	1737 (100)	1782 (102)	1823 (105)	1787 (103)

X

X

Table A6.4.1.1-3 Group mean test material intake (mg/kg bw/d)

Week	Dose level (ppm)									
	Males					Females				
	0	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
1-13	-	0.07	0.33	1.93	19.87	-	0.07	0.37	2.28	24.03

Table A6.4.1.1-4 Group mean haematology

Week	Dose level (ppm)									
	Males					Females				
	0	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
MCHC (%)	35	35	35**	35*	34	36	36	35	36	36
PCV (%)	46	46	46	45	45	45	44	44	44*	43***
MCV (pg)	52	~~5~~ 51	52	52	52	54	53	54	53	51***
Platelet count (1000/cmm)	852	858	911	948*	926	913	937	933	993	1028*
Prothrombin time (secs)	15.0	15.8*	14.7	15.2	14.7	14.2	14.4	14.0	13.7*	13.5**

X

Statistical analysis: * p≤0.05 ** p≤0.01 *** p≤0.001

Table A6.4.1.1-5 Results Group mean clinical chemistry changes

Week	Dose level (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Total protein (g%)	6.8	6.9	7.1**	7.1**	7.4***	7.2	7.8*	7.6*	7.8**	7.9**
α1 globulin (g%)	1.3	1.3	1.5*	1.5**	1.7***	1.1	1.1	1.2	1.3*	1.4***
α2 globulin (g%)	0.4	0.4	0.4	0.4	0.5***	0.4	0.5**	0.4*	0.5**	0.6***
β globulin (g%)	1.7	1.6	1.8	1.6	2.0**	1.4	1.6*	1.6	1.7**	1.8***
A:G ratio (-:1)	0.8	0.9	0.7	0.8	0.6**	1.1	1.0	1.1	1.0	0.9***
ALT (iu/l)	34	31	30	28	32	30	28	27	24*	24*
AST (iu/l)	73	63	63	61*	71	74	59*	53***	40***	48***
Glucose (mg%)	140	132	127	135	146	125	136	140*	151***	144**

Statistical evaluation: * p≤0.05 ** p≤0.01 *** p≤0.001

Table A6.4.1.1-6 Results Group mean liver and thyroid weights

Weight	Dose level (ppm)									
	Males					Females				
	0	1	5	30	300	0	1	5	30	300
Terminal body weight g	540.5	563.9	540.3	546.8	539.1	304.9	324.6	330.6	325.8	298.0
Absolute thyroid weight, g	0.024	0.024	0.025	0.030	0.048**	0.019	0.019	0.021	0.023*	0.032**
Bodyweight relative thyroid weight,%	0.0044	0.0042	0.0046	0.0054	0.0091**	0.0061	0.0059	0.0063	0.0071	0.107**
Absolute liver weight, g	19.1	21.0	19.4	21.8	27.2**	10.8	11.3	12.7*	13.4**	16.6**
Bodyweight relative liver weight,% (% of controls)	3.54 (100)	3.72 (105)	3.59 (101)	3.99* (113)	5.05** (143)	3.52 (100)	3.48 (99)	3.86 (110)	4.13** (117)	5.57** (158)

Statistical analysis: * p≤0.05 ** p≤0.01 *** p≤0.001

X

Table A6.4.1.1-7: Group incidence of histopathological findings

Finding	Dose level (ppm)									
	Males					Females				
	0	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
Thyroid										
-follicular epithelial hypertrophy	3	1	0	5	8	1	0	0	0	10***
-follicular cell hyperplasia	2	0	0	1	6	0	0	1	1	2
Liver										
-Panacinar fatty vacuolation(ORO)	0	2	0	1	7**	0	0	0	0	1
-Centriacinar fatty vacuolation	4	3	~~1~~ 2	6	3	7	9	6	10	7

Number examined: 10/sex/group; ORO: oil red O staining
Statistical analysis: ** $p < 0.01$; *** $p < 0.001$

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.4.2 Body weight: Each rat was weighed on the day that treatment commenced, at weekly intervals throughout the treatment period and before necropsy. 3.4.8 Urinalysis: *Parameters:* nitrite 3.5.2 Gross and histopathology: Epididymides, femoral bone and articular surface, pituitary, rectum, sciatic nerve, seminal vesicles, skeletal muscle, sternum with marrow, vagina. 3.5.3 Other examinations: Neurological examinations was performed after 12 weeks of treatment on all animals from controls and top dose group : cranial nerve reflexes, segmental reflexes, postural reactions and general observations were performed.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.2 Body weight gain: *At 30 ppm, male body weight gain was slightly reduced in Week 1 and, at 300 ppm, body weight gain was markedly reduced during the first week of treatment.* During the subsequent 11 weeks the bodyweight gains of males receiving 300 ppm was significantly higher and that of the other treated groups were similar or superior to that of their respective controls. *However, the overall weight gain of females at this high dose level was only slightly lower than controls* ~~*and that of males was similar to controls*~~ although this difference was not statistically significant. The overall bodyweight gains of other treated females were superior to those of their controls, there was no relationship to dosage and this difference is probably fortuitous. The overall weight gains in treated males were similar to those of the controls. The fluctuations in bodyweights recorded during week 13 are considered to be attributable to the effects of clinical pathology investigations at this time, although it should be noted that the bodyweight of males receiving the high treatment level displayed the most marked depression. 4.3 Food consumption and compound intake: The low food intakes noted for all groups for week 13 of treatment were attributable to the effects of clinical pathology procedures performed during this week. 4.6.1 Organ weights: ~~*Body weight relative weights were increased in both sexes at 30 and 300 ppm.*~~ When bodyweight-relative values were considered higher liver weights were noted in males and females which received 30 or 300 ppm. The absolute and bodyweight-relative salivary gland weights of treated females tended to be lower than those of their controls, although statistical significance was not consistently attained. However, as there was no evidence of dosage-relationship and no associated histopathological changes were noted, this was

	considered to be a change occurrence and toxicological significance was not attached.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods : *Blood samples for hematology and clinical chemistry evaluations*, and urine samples *were taken from all animals ~~during~~* after *Week 12~~ whilst urine samples were collected from all rats in Week 13.~~* 5.2 Results and discussion: ~~*At dose levels of up to 5 ppm*~~ In animals receiving up to 30 ppm, *plasma changes were generally minor and not associated with any histopathological change.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.4.1/02** XXXX M&B 46030: Toxicity by oral (capsule) administration to Beagle dogs for 13 weeks. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 82-1 (1984) Also compliant with EU 88/302/EEC, B.27 (1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Extra specific neurological examinations were also conducted	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Pure bred Beagle	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: approx. 19 to 23 weeks Weight: 8.0 – 9.8 kg (males) and 7.3– 9.5 kg (females)	
3.2.6	Number of animals per group	8 – 4 males and 4 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral capsule	
3.3.1	Duration of treatment	90 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Post exposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	Gavage: gelatine capsule	
3.3.4.2	Concentration	0.5, 2, and 10 mg/kg bw	
3.3.4.3	Vehicle	None	
3.3.4.4	Concentration in vehicle	n.a.	
3.3.4.5	Total volume applied	n.a.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
3.3.4.6 Controls	Blank gelatine capsules	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: daily In addition a detailed weekly examination was performed on each animal, at which conditions of a chronic nature were recorded. Each animal was subjected to a rigorous veterinary examination before dosing commenced and after three, seven and 11 weeks of treatment. A neurological examination was conducted on each animal before treatment started and after 6 and 12 weeks of treatment.	**x**
3.4.1.2 Mortality	Yes: daily	
3.4.2 Body weight	Yes: weekly	
3.4.3 Food consumption	Yes: daily	
3.4.4 Water consumption	Yes: daily	
3.4.5 Ophthalmoscopic examination	Yes: 6 and 12 weeks	
3.4.6 Haematology	Yes Number of animals: all animals Time points: 6 and 12 weeks Parameters: packed cell volume, haemoglobin concentration, erythrocyte count, reticulocyte count total and differential leukocyte count, blood film, platelet count, mean cell haemoglobin, mean cell volume, mean cell haemoglobin concentration, , prothrombin time and thromboplastin time.	**x**
3.4.7 Clinical chemistry	Yes Number of animals: all animals Time points: 6 and 12 weeks Parameters: sodium, potassium, chloride calcium and inorganic phosphorus, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, electrophoretic protein fractions, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase	**x**
3.4.8 Urinalysis	Yes Number of animals: all animals Time points: 6 and 12 weeks Parameters: appearance, volume,, specific gravity, pH, protein, glucose, blood, ketones, bilirubin, urobilingogen, nitrite	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes All dose groups Organs: liver, kidneys, adrenals, testes, uterus with cervix, ovaries, spleen, brain, heart, lungs, pituitary, prostrate with urethra sample, thyroid with parathyroid, thymus	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
3.5.2 Gross and histopathology	Yes All dose groups Macroscopic pathology: external features and orifices, neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, bone marrow, skin, eyes	x
3.5.3 Other examinations		
3.5.4 Statistics	The significance of inter-group differences in bodyweight change, blood composition and quantitative urinalysis was assessed by Student's t test using pooled error variance. For organ weights, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used	
3.6 Further remarks		
4.1 Observations 4.1.1 Clinical signs	**4. RESULTS AND DISCUSSION** Clinical signs were generally confined to animals administered the highest dose of 10 mg/kg bw/d. Signs indicative of general toxicity were particularly marked during the first two or three weeks of treatment and included inappetence, emaciation, underactivity and hunched posture. However, significant inappetence was seen only in one female during Weeks 11 and 12 due to a gradual recovery. In addition, signs of possible neurological disturbance (tremors and/or convulsions and/or head nodding) were noted during the first seven weeks of treatment. One female exhibited a convulsive episode. At 2 mg/kg bw/d, inappetence in two females during Week 2 or Weeks 1-4 was the only clinical sign. <u>Veterinary examination</u> At 10 mg/kg bw/d, during unscheduled examinations, convulsive episodes were recorded in one male in Week 8 and in another male in Week 13. In addition, in Week 2, one female exhibited subdued behaviour, ataxia, convulsions, tremors, head nodding and facial twitching over a two-day period. Routine examinations after three weeks of treatment revealed emaciation and occasional muscular twitching of the whole body in one male given 10 mg/kg bw/d. <u>Neurological examination</u> After six weeks of treatment, one male dosed with 10 mg/kg bw/d showed head nodding, facial twitching and exaggerated blink and gag responses. After 12 weeks, depressed tactile placing response was seen in the surviving female at this dose level.	x

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
4.1.2 Mortality	One male and three females given 10 mg/kg bw/d were killed during the second week of treatment. The male was killed on Day 10 following inappetence, bodyweight loss and emaciation. Slight dehydration and an abnormal temperature was also evident. The three females killed during Week 2 also showed inappetence and weight loss. In addition, one female exhibited emaciation, subdued behaviour, slight dehydration, excessive salivation and hindlimb extension. Two other females displayed disorientation, ataxia, limb jerks, apparent lack of vision/awareness and an irregular heart rate, and were killed after a series of convulsions. Increased red cell parameters (packed cell volume, hemoglobin concentration and red blood cell count), lowered prothrombin and activated partial thromboplastin times were found in these animals at necropsy. These findings were considered to be due to hemoconcentration because of poor health. Macroscopic examination confirmed their emaciated state but there were no treatment-related changes in appearance or organ weights.	
4.2 Body weight gain	At 10 mg/kg bw/d, two males and three females exhibited bodyweight loss on Day 7. Bodyweight for the other two males was static during Week 1. Ill-health and inappetence resulted in further loss of weight in one male and all three females killed during Week 2. The other male, which had displayed weight loss until Day 21, regained weight from Day 28 and reached its initial bodyweight by Day 35. Overall bodyweight gain of surviving animals was similar to controls. At 2 mg/kg bw/d, transitory weight loss or stasis was noted in two females during Week 2. The overall bodyweight gains of dogs given 0.5 or 2 mg/kg bw/d were similar to the control values.	**x**
4.3 Food consumption and compound intake	At 10 mg/kg bw/d, food consumption was markedly lower than in controls during the first week of treatment and inappetence was apparent from Day 1 in all except one male. Consumption was unaffected at 2 mg/kg bw/d except in two females which showed a slight decrease in Week 2. No treatment-related effect was seen at 0.5 mg/kg bw/d.	**x**
4.4 Ophthalmoscopic examination	no effects	
4.5 Blood analysis		
4.5.1 Haematology	There were no haematological changes that could be unequivocally attributed to treatment. The examinations after 6 and 12 weeks revealed minimally higher red cell parameters (packed cell volume, haemoglobin concentration and erythrocyte count) for one male receiving 10 mg/kg bw/d an after 12 weeks for two females 2.0 mg/kg bw/d. All values, however, were considered to be within the range of expectancy.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
4.5.2 Clinical chemistry	At 10 mg/kg bw/d, high alkaline phosphatase activity and low cholesterol were found in males at Weeks 6 and 12. In addition, in Week 6, aspartate aminotransferase in males dosed with 10 mg/kg bw/d and plasma urea in females given 2 mg/kg bw/d were higher than in controls. However, these minor transitory inter-group differences were considered to be of questionable toxicological significance. Other differences were considered to be fortuitous.	
4.5.3 Urinalysis	No effects	
4.6 Sacrifice and pathology		
4.6.1 Organ weights	Organ weight analysis for animals killed after 13 weeks revealed for one male which received 10 mg/kg bw/d, higher absolute and bodyweight-relative spleen weight and higher absolute adrenal weights; a higher absolute and bodyweight-relative thymus weight was evident for this animal and for one other male of this group. However these differences were not clearly apparent when the group mean values were considered. Organ weights for the surviving female receiving 10 mg/kg bw/d and animals receiving 0.5 or 2.0 mg/kg bw/d were considered to be unaffected by treatment.	
4.6.2 Gross and histopathology	Macropathological examination of animals killed after 13 weeks did not reveal any significant finding. The only changes that could be related to treatment were follicular and parafollicular atrophy of the mesenteric lymph nodes and cortical atrophy of the thymus in one male which received 10 mg/kg bw/d and cortical atrophy of the thymus in one female given the same dose. These were both decedent animals and the changes observed were more likely to be caused by stress than as a direct result of treatment. Other findings were considered to be normal for beagle dogs of this age.	
4.7 Other	None	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and methods	Groups of 4 male and 4 female pure-bred Beagle dogs were given a single daily oral dose, by gelatin capsule, of 0, 0.5, 2 or 10 mg/kg bw/d of Fipronil for thirteen consecutive weeks. The quantity of test material administered was adjusted according to the most recent bodyweight. At the start of the study, the dogs were approx. 19 and 23 weeks of age; the males weighed between 8.0 and 9.8 kg and the females weighed between 7.3 and 9.5 kg. Animals were observed daily for mortality and clinical signs. A detailed weekly examination was also performed. Furthermore, each animal was subjected to a rigorous veterinary examination prior to the start of dosing and after 3, 7 and 11 weeks of treatment. Neurological examinations were conducted prior to the start of treatment and after 6 and 12 weeks of dosing. They comprised assessments of reflexes, reactions and general observations (cranial reflexes: pupillary, palpebral, gag, general examination of head for neural function; segmental reflexes: flexor, patellar, extensor tone; postural reactions: placing, righting, hopping, extensor postural thrust and tonic neck reactions; general observations of behavioural changes, gait abnormalities and tremors or other dyskinesias). The eyes of each dog were examined for ophthalmological abnormalities prior to the start of dosing and after 6 and 12 weeks of treatment. Individual bodyweights and food intake was recorded weekly. Blood samples for hematology and clinical chemistry were taken prior to the start of treatment and after 6 and 12 weeks before dosing and following overnight starvation. Urine samples were collected overnight at similar time points during deprivation of food and water. Each animal was examined externally and internally (contents of the cranial, thoracic, abdominal and pelvic cavities) for macroscopic changes. Selected organs were weighed and a comprehensive range of tissues preserved and examined microscopically.	x
5.2 Results and discussion	<u>Mortality</u> One male and three females given 10 mg/kg bw/d were killed during the second week of treatment. The male was killed on Day 10 following inappetence, bodyweight loss and emaciation. Slight dehydration and an abnormal temperature was also evident. The three females killed during Week 2 also showed inappetence and weight loss. In addition, one female exhibited emaciation, subdued behaviour, slight dehydration, excessive salivation and hindlimb extension. Two other females displayed disorientation, ataxia, limb jerks, apparent lack of vision/awareness and an irregular heart rate, and were killed after a series of convulsions.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
	Increased red cell parameters (packed cell volume, hemoglobin concentration and red blood cell count), lowered prothrombin and activated partial thromboplastin times were found in these animals at necropsy. These findings were considered to be due to hemoconcentration because of poor health. Macroscopic examination confirmed their emaciated state but there were no treatment-related changes in appearance or organ weights. Clinical signs Clinical signs were generally confined to animals administered the highest dose of 10 mg/kg bw/d. Signs indicative of general toxicity were particularly marked during the first two or three weeks of treatment and included inappetence, emaciation, underactivity and hunched posture. Apparently due to a gradual recovery, by weeks 11-12 inappetence was the only significant clinical sign that was noted in one female. In addition, signs of possible neurological disturbance (tremors and/or convulsions and/or head nodding) were noted during the first seven weeks of treatment. One female exhibited a convulsive episode. At 2 mg/kg bw/d, inappetence in two females during Week 2 or Weeks 1-4 was the only clinical sign. No other treatment-related clinical signs were seen. Veterinary examination At 10 mg/kg bw/d, during unscheduled examinations, convulsive episodes were recorded in one male in Week 8 and in another male in Week 13. In addition, in Week 2, one female exhibited subdued behaviour, ataxia, convulsions, tremors, head nodding and facial twitching over a two-day period. Routine examinations after three weeks of treatment revealed emaciation and occasional muscular twitching of the whole body in one male given 10 mg/kg bw/d. Neurological examination After six weeks of treatment, one male dosed with 10 mg/kg bw/d showed head nodding, facial twitching and exaggerated blink and gag responses. After 12 weeks, depressed tactile placing response was seen in the surviving female at this dose level. Bodyweight At 10 mg/kg bw/d, two males and three females exhibited bodyweight loss on Day 7. Bodyweight for the other two males was static during Week 1. Ill-health and inappetence resulted in further loss of weight in one male and all three females killed during Week 2. The other male, which had displayed weight loss until Day 21, regained weight from Day 28 and reached its initial bodyweight by Day 35. Overall bodyweight gain of surviving animals was similar to controls. At 2 mg/kg bw/d, transitory weight loss or stasis was noted in two females during Week 2. The overall bodyweight gains of dogs given 0.5 or 2 mg/kg bw/d were similar to the control values.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**	
	Food consumption At 10 mg/kg bw/d, food consumption was markedly lower than in controls during the first week of treatment and inappetence was apparent from Day 1 in all except one male. Consumption was unaffected at 2 mg/kg bw/d except in two females which showed a slight decrease in Week 2. No treatment-related effect was seen at 0.5 mg/kg bw/d. Ophthalmoscopy There were no treatment-related changes. Hematology None of the changes were attributed to treatment since all values were within the historical control range. Clinical chemistry At 10 mg/kg bw/d, high alkaline phosphatase activity and low cholesterol were found in males at Weeks 6 and 12. In addition, in Week 6, aspartate aminotransferase in males dosed with 10 mg/kg bw/d and plasma urea in females given 2 mg/kg bw/d were higher than in controls. However, these minor transitory inter-group differences were considered to be of questionable toxicological significance. Other differences were considered to be fortuitous. Urinalysis There were no treatment-related findings.	x
	Organ weights None of the differences in organ weights was considered to be of toxicological significance.	X
	Macroscopic pathology No macroscopic treatment-related changes were observed.	
	Histopathology The only treatment-related finding was follicular and parafollicular atrophy of the mesenteric lymph nodes and cortical atrophy of the thymus in one male given 10 mg/kg bw/d. Cortical atrophy of the thymus was also seen in one female at this dose level. Since both of the affected animals were decedents, these findings were probably caused by stress rather than a direct effect of treatment with Fipronil.	
5.3 Conclusion	Oral (capsular) administration of 10 mg/kg bw/d to Beagle dogs for 13 weeks was toxic causing inappetence, loss of bodyweight and neurological effects on the central nervous system; one male and three female dogs with poor general state were killed for humane reasons. In surviving animals, there were no neurological symptoms seen toward the end of the treatment period, indicating a degree of adaptation to treatment. The NOAEL was established at 0.5 mg/kg bw/d based on slight, transitory inappetence and slight depression of weight gain in two females at 2 mg/kg bw/d.	
5.3.1 LO(A)EL	2.0 mg/kg bw/d	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**Subchronic oral toxicity test in dogs (90-day study)**

5.3.2 NO(A)EL	0.5 mg/kg bw/d
5.3.3 Other	
5.3.4 Reliability	1
5.3.5 Deficiencies	No

Table A6.4.1.2-1: Group mean bodyweight and bodyweight change (kg)

Days	Dose level (mg/kg bw/d)							
	Males				Females			
	0	**0.5**	**2.0**	**10**	**0**	**0.5**	**2.0**	**10**
	Bodyweight							
0	9.0	9.1	9.2	8.8	8.1	8.6	8.0	7.9
7	9.4	9.5	9.5	8.3	8.5	9.0	8.3	7.6
14	9.5	9.7	9.8	8.4	8.6	9.2	8.3	8.3*
21	10.0	10.2	10.2	8.7	9.2	9.7	8.8	8.9*
35	10.4	10.7	10.8	9.6	9.5	10.0	9.2	8.6*
70	11.1	11.5	11.5	10.8	10.2	10,7	9.9	9.6*
	Bodyweight Change							
0-91 (% of control)	2.4 (100)	2.7 (113)	2.6 (108)	2.6 (108)	2.4 (100)	2.3 (96)	2.0 (83)	2.1 (88)

*data for one animal only

Table A6.4.1.2-2: Group mean food consumption (g/animal/week)

Week	Dose level (mg/kg bw/d)							
	Males				Females			
	0	**0.5**	**2.0**	**10**	**0**	**0.5**	**2.0**	**10**
-1	2790	2800	2800	2770	2560	2670	2600	2600
1	2770	2800	2790	1870	~~2560~~ 2650	2760	2560	1820
2	2790	2800	2780	1670	2610	2740	2460	2800
3	2750	2800	2800	2130	2710	~~20800~~ 2800	2670	2800
Total Weeks 1-13 (% of control)	36.2 (100)	36.4 (101)	36.4 (101)	32.6 (91)	35.0 (100)	36.0 (103)	33.9 (97)	33.1 (95)

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.4.1.1 Clinical signs: *A neurological examination was conducted on each animal before treatment started and after 6 and 12 weeks of treatment:* Reflexes tested and observations performed included: cranial nerve reflexes, segmental reflexes, postural reactions and general observations. 3.4.7 Clinical chemistry: *Parameters*: Creatine phosphokinase activity 3.5.2 Gross and histopathology: ~~*Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, bone marrow, skin, eyes*~~ Histopathology: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes peripheral nerve, skin, eyes, femoral bone and articular surface, skeletal muscle, sciatic nerve, sternal bone and marrow, vagina, epididymides.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1.1 Clinical signs: ~~*However, significant inappetence was seen only in one female during Weeks 11 and 12 due to a gradual recovery.*~~ Apparently due to a gradual recovery, by weeks 11-12 inappetence was the only significant clinical sign that was noted in one female. 4.2 Body weight gain: See table A6.4.1.2-1 4.3 Food consumption and compound intake/5.2 Results and discussion: See table A6.4.1.2-2 *Consumption was unaffected at 2 mg/kg bw/d except in two females which showed a slight decrease in Week 2* for the first and during the first four weeks of treatment for the second female.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Urine samples were collected overnight at similar time points during deprivation of food and water,* prior to the start of treatment and after 6 and 12 weeks before dosing. 5.2 Results and discussion: *Organ weights*: ~~*None of the differences in organ weights was considered to be of toxicological significance.*~~ Organ weight analysis for animals killed after 13 weeks revealed for one male which received 10 mg/kg bw/d, higher absolute and bodyweight-relative spleen weight and higher absolute adrenal weights; a higher absolute and bodyweight-relative

	thymus weight was evident for this animal and for one other male of this group. However these differences were not clearly apparent when the group mean values were considered. Organ weights for the surviving female receiving 10 mg/kg bw/d and animals receiving 0.5 or 2.0 mg/kg bw/d were considered to be unaffected by treatment.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.4.1/03** XXXX M&B 46030: Toxicity by oral (capsule) administration to Beagle dogs for 52 weeks. XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 82-1 (1984) Also compliant with EU 88/302/EEC, B.30	
2.2	**GLP**	Yes	
2.3	**Deviations**	Extra specific neurological examinations and perfusion fixation for selected animals were also conducted	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	PGS963	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	95.4%	X
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Dog	
3.2.2	Strain	Pure bred Beagle	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: approx .20 to 23 weeks Weight: 7.5 – 9.1 kg (males) and 6.3– 8.3 kg (females)	
3.2.6	Number of animals per group	12 – 6 males and 6 females	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral capsule	
3.3.1	Duration of treatment	52 weeks	
3.3.2	Frequency of exposure	Daily	
3.3.3	Post exposure period	None	
3.3.4	**Oral**		
3.3.4.1	Type	Gavage: gelatine capsule	
3.3.4.2	Concentration	Dose: 0.2, 2, and 5 mg/kg bw/d	
3.3.4.3	Vehicle	The test material was weighed directly in to the capsules for the first fifteen days but, from Day 16 was added to the capsules as a 1 in 20 mixture of Fipronil: lactose	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
3.3.4.4 Concentration in vehicle	n.a.	
3.3.4.5 Total volume applied	n.a.	
3.3.4.6 Controls	Blank capsules	x
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: daily Each animal was subjected to veterinary examination before dosing commenced and after 11, 23, 35 and 47 weeks of treatment. Neurological examination: Before treatment started and after 12, 24, 38 and 50 weeks	
3.4.1.2 Mortality	Yes: daily	
3.4.2 Body weight	Yes: weekly	
3.4.3 Food consumption	Yes: daily	
3.4.4 Water consumption	Yes: daily	
3.4.5 Ophthalmoscopic examination	Yes: 12, 24 and 50 weeks	
3.4.6 Haematology	Yes Number of animals: all animals Time points: 12, 24 and 50 weeks Parameters: Packed cell volume, haemoglobin concentration, erythrocyte count, reticulocyte count, total and differential leukocyte count, blood film, platelet count, mean cell haemoglobin, mean cell volume, mean cell haemoglobin concentration, prothrombin time and thromboplastin time.	
3.4.7 Clinical chemistry	Yes Number of animals: all animals Time points: 12, 24 and 50 weeks Parameters: sodium, potassium, chloride calcium and inorganic phosphorus, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, electrophoretic protein fractions, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,	x
3.4.8 Urinalysis	Yes Number of animals: all animals Time points: 11, 23 and 48 weeks Parameters: appearance, volume,, specific gravity, pH, protein, glucose, blood, ketones, bilirubin, urobilingogen, nitrite	
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes all dose groups Organs: liver, kidneys, adrenals, testes, uterus with cervix, ovaries, spleen, brain, heart, lungs, pituitary, prostrate with urethra sample, thyroid with parathyroid, thymus	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
3.5.2 Gross and histopathology	Yes all dose groups Macroscopic pathology: external features and orifices, neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, nerve, bone marrow, skin, and eyes.	x
3.5.3 Other examinations		
3.5.4 Statistics	Standard deviations were calculated, as considered appropriate, using the sample statistic. The significance of inter-group differences in bodyweight gain, haematology, blood chemistry and urinalysis was assessed by Student's t-test using a pooled error variance. For organ weights, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. Inter-group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test.	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Observations 4.1.1 Clinical signs	Signs indicative of possible neurological disturbance occurred intermittently from Week 2 in dogs s receiving 5.0 mg/kg bw/d. These signs included convulsions, twitching or tremors of various muscle beds, nervous behaviour and other abnormalities of gait and posture. Overall, evidence of a response to treatment was noted in all the dogs receiving the high dosage and in five males and three females receiving 2.0 mg/kg bw/d. Convulsive episodes wee observed in one male and one female receiving 2.0 mg/kg bw/d and two males receiving 5.0 mg/kg bw/d. A convulsion was noted before dosing on Day 18 of treatment for one male receiving 2.0 mg/kg bw/d. Recovery was rapid and the animal appeared normal within five minutes. In Week 11, although a convulsive episode was not observed, the animal displayed signs suggesting it had convulsed. Marked signs of ill-health, including bodyweight loss and inappetence, were noted over the subsequent three days and thin animal was killed on Day 76. During Week 45 one female receiving 2.0 mg/kg bw/d had a convulsion, recovery appeared complete within 30 minutes. This dog also appeared to be in a pre- or post- convulsive state on one day during Week 47.	x
	One male receiving 5.0 mg/kg bw/d convulsed on one occasion in Week 27. A further series of convulsions commencing 15 minutes after dosing and other severe signs of reaction to treatment, which included an apparent impairment of vision, were noted during a three-day period in Week 31. The dog was killed at the end of this period.	x

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
	When dogs are lifted, there is often some rigidity of the limbs. An exaggeration of this reaction was noted during Week 32 for one female receiving 5.0 mg/kg bw/d. This sign was subsequently observed with increasing incidence and persistence in animals receiving 2.0 or 5.0 mg/kg bw/d but was not apparent for animals receiving 0.2 mg/kg bw/d. Nervous behaviour was also noted on isolated occasions during the treatment in some animals, particularly those receiving 5.0 mg/kg bw/d and females receiving 2.0 mg/kg bw/d, although no clear evidence of an increase in this sign was obtained from the routine examinations of the animals. From Week 2 a range of abnormalities of stance and gait occurred occasionally for males receiving 5.0 mg/kg bw/d. The signs observed included ataxia, unsteady gait and stiffness of the limbs. Similar findings were also observed in two females receiving this dosage and in one male and two females receiving 2.0 mg/kg bw/d. Periods of muscular twitching or tremor were noted in two males and two females receiving 2.0 mg/kg bw/d and three males and two females receiving 5.0 mg/kg bw/d. The head, pinnae, shoulders or hindlimbs were most frequently affected, although on some occasions the whole of the body was involved. A number of other signs considered to probably associate with treatment was noted for dogs receiving 2.0 or 5.0 mg/kg bw/d. These included changes in activity patterns, vocalisation, head nodding, aggression and resistance to dosing. There was no clear effect of treatment in animals receiving 0.2 mg/kg bw/d. One female was markedly overactive during Weeks 13 to 17. It developed lesions on the forepads and tail from continuous pacing around the pen. In Weeks 18 to 19 the animal was, however, underactive while thin build was apparent during Weeks 13 to 39. Bodyweight loss between Weeks 12 and 15 was considered to associate with the behavioural change and a modification of the feeding regime was introduced. No similar changes in behaviour were noted for the other treated animals and it was concluded that this change was unlikely to have been associated with treatment.	**x**
4.1.2 Mortality	Following severe reaction to treatment, one male receiving 2.0 mg/kg bw/d and two males receiving 5.0 mg/kg bw/d were killed in Weeks 11, 31 and 34 respectively	
4.2 Body weight gain	During the first 26 weeks the weight gain of one female receiving 5.0 mg/kg bw/d was particularly low. This affected the mean bodyweights for the group over this period. The growth performance of other treated dogs was similar to that of their controls. Bodyweight losses were noted in the periods just before sacrifice for animals killed during the study.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
4.3 Food consumption and compound intake	Throughout the study the food intake of treated animals was generally similar to that of their controls. The clinical signs noted above occasionally associated with periods of inappetence. The feeding regimen of affected animals was amended by addition of water to the basal diet to enhance palatability with, if appropriate, provision of meat supplement. In addition, the amount of diet provided to each animal was increased to 60 g per day during Weeks 16 and 18. This change was introduced to maintain consistency with the female receiving 0.2 mg/kg bw/d because of its marked overactivity and weight loss	x
4.4 Ophthalmoscopic examination	no effects	
4.5 Blood analysis		
4.5.1 Haematology	Investigation after 50 weeks revealed, when compared with the controls, slightly high packed cell volume, haemoglobin concentration and red blood cell counts among females receiving 2.0 or 5.0 mg/kg bw/d. These parameters were also slightly high on this occasion in one high dose male. The change was minor and not considered to be toxicologically significant. Throughout the treatment period, the haematological profile of the other groups was unaffected by treatment. Other inter-group differences that attained statistical significance were minor or lacked dosage relationship and were not attributed to treatment	x
4.5.2 Clinical chemistry	Blood chemistry investigations after 12, 25 and 50 weeks of treatment revealed a number of inter-group differences that attained a level of statistical significance when compared with the controls. However these changes were small, generally without trend and of a transitory nature. They were, therefore, considered to be of no toxicological significance. Analysis of the blood samples taken ante mortem from animals killed during the treatment period showed, when compared with pre-treatment values, slightly high plasma alanine and aspartate amino transferase activities for both decedent animals receiving 5.0 mg/kg bw/d and marginally high plasma levels of urea, total bilirubin and cholesterol for the decedent dog receiving 2.0 mg/kg bw/d.	
4.5.3 Urinalysis	No effects	
4.6 Sacrifice and pathology		

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
4.6.1 Organ weights	Analysis of organ weights for animals killed after 52 weeks showed few inter-group differences, and none were considered to be directly related to treatment. Bodyweight-relative heart weights were slightly higher than controls in females that had received 2.0 or 5.0 mg/kg bw/d. Absolute values were unaffected and the inter-group difference was, therefore, attributed to the lower bodyweights recorded for these treated dogs rather than to a direct effect upon the organ. The slightly high mean spleen weight in females receiving 5.0 mg/kg bw/d reflected the high value recorded for one animal. The weight recorded for this animal was, however, with the background range. High thyroid weight and slightly high liver weight were noted for one dog given 5.0 mg/kg bw/d but there was no similar change in the other animals. These differences, therefore, were not attributed to treatment. Among animals killed during the treatment period two, one each from the high and intermediate dose groups had high spleen weights.	
4.6.2 Gross and histopathology	Macropathological examination of animals killed during the treatment period or after 52 weeks did not show any findings that were considered to be related to treatment. There were no histopathological findings considered to be related to treatment in animals killed during the treatment period or after 52 weeks.	
4.7 Other	**Veterinary examination**: Routine veterinary examinations performed during the course of the treatment period showed a number of abnormalities in dogs receiving 2.0 or 5.0 mg/kg bw/d that were considered to relate to treatment. The findings included tense, nervous and excitable behaviour and abnormal stiffness or positioning of hindlimbs. In addition, twitching of the facial muscles and hyperaesthesia were also noted. These changes were, in general, observed in a minority of dogs at each dose level. No treatment-related changes were noted for dogs receiving 0.2 mg/kg bw/d.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
	Veterinary examination ante mortem of the three dogs killed during the treatment period confirmed their poor condition and indicted central nervous system impairment. Abnormalities included splayed hindlimbs and other stance abnormalities, abnormal gait, twitching of the muscles of the head and, in one male treated at 5.0 mg/kg bw/d , apparent visual impairment. **Neurological examination:** The treatment-related effects noted during the routine veterinary examinations were generally confirmed at the detailed neurological investigations performed during the treatment period. The changes comprised: tenseness; abnormalities of gait or stance; exaggeration of the hopping and gag reflexes. Overall a clear response to treatment was noted in three males and five females receiving the high dosage and two females receiving the intermediate dosage. Tenseness was generally noted in three females receiving 5.0 mg/kg bw/d and, from Week 25 of treatment, in two females receiving 2.0 mg/kg bw/d. High incidences of tenseness were also noted after 12 weeks in males receiving 5.0 mg/kg bw/d and after 38 weeks in males receiving 2.0 mg/kg bw/d. However, in view of the sporadic nature of this finding in treated males and its occasional occurrence in the controls, a definite association with treatment can not be made for males. Abnormal stance (an excessive posterior extension of the hindlimbs), was noted occasionally from Week 25 for two males receiving 5.0 mg/kg bw/d and from Week 39 in three females receiving this dosage. This finding frequently associated with depression of other responses of the hindlimbs. Among animas receiving 5.0 mg/kg bw/d exaggeration of the hopping reflex was noted after 12, 38 and 50 weeks in one male and after Weeks 24 and 38 in two females. A foot sliding test performed in Weeks 25 and 39 indicated a depressed reaction on both occasions in two males and two females receiving 5.0 mg/kg bw/d; a further male receiving 5.0 mg/kg bw/d was affected at Week 39. An exaggerated gag reflex was noted after 50 weeks in three females receiving the high dosage. Examination ante mortem of the male receiving 5.0 mg/kg bw/d killed in week 31 showed an abnormal stance and gait, poor visual and tactile placing reflexes, poor menace reaction and twitching of the ears. The other male of this dose group that was killed had abnormalities of many cranial and segmental reflexes, and an abnormal gait. A detailed assessment ante mortem of the male receiving 2.0 mg/kg bw/d killed in Week 11 of treatment was not possible in view of its poor health status.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
5.1 Materials and methods	Groups of 6 male and 5 female pure-bred Beagle dogs were given a single daily oral dose by capsule, of 0, 0.2, 2 or 5 mg/kg bw/d of Fipronil for 52 consecutive weeks. The dogs were about 20 to 23 weeks of age at the start of dosing and weighed between 7.5 and 9.1 kg (males) and between 6.3 and 8.3 kg (females). They were housed individually and were acclimatised for 4 weeks. The test material was weighed directly into the capsules for the first fifteen days but from Day 16 was added to the capsules as a 1 in 20 mixture of Fipronil:lactose to enhance accuracy of dose administration. Controls received either empty capsules or 100 mg/kg bw/d of lactose alone, equivalent to the quantity of the admixture given to the high dose animals. The daily doses were administered after feeding. Homogeneity of mixing Fipronil with lactose and its stability in this carrier were assessed following storage for 3, 8, 14 and 35 days at 21°C. The achieved concentration of the test material in the admixture was checked in the batches used in Weeks 3, 4, 6, 10, 18, 26, 34, 42 and 50 of treatment. The content of Fipronil in the capsules containing this alone (used during the first 15 days of dosing) was also checked. Animals were observed daily for mortality and clinical signs, especially before and after dosing. A veterinary examination was conducted on each dog 6 days prior to the start of dosing and after 11, 23, 35 and 47 weeks of treatment. In addition, a neurological examination was performed 6 days before the start of dosing and after 12, 24, 38 and 50 weeks. This comprised evaluation of reflexes, reactions and general observations (cranial reflexes: direct and indirect pupillary light, palpebral, gag and general examination of head for cranial nerve function; segmental reflexes: flexor withdrawal, patellar and crossed extensor; postural reactions: placing, extensor postural thrust, righting, hopping and tonic neck; general observations gait and stance abnormalities, behavioural changes and presence of tremors or other dyskinesias). Individual bodyweights were recorded weekly whilst food consumption was documented each day. Water intake was checked by visual inspection. Ophthalmic examinations were performed prior to the start of dosing and after 12, 24 and 50 weeks. Blood samples for hematology and clinical chemistry were also taken at these time points. Urine samples were collected from each dog six days before the start of treatment and after 11, 23 and 48 weeks. After 50 and 51 weeks of treatment, blood samples were obtained from each surviving dog 23 hours after dosing. Anticoagulant was not used for samples taken after 51 weeks. Plasma and serum were separated as appropriate and stored frozen.	x

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
5.2 Results and discussion	At necropsy, all animals were subjected to external and internal examination (cranial, thoracic, abdominal and pelvic cavities and their viscera) for macroscopic changes. Two males and two females from each group (only one male from the highest dose level) were perfusion-fixed under anaesthesia for a detailed examination of the brain, spinal cord and requisite nerves. The other tissues were also preserved. The remaining dogs in each group were also examined externally and internally, selected organs were weighed and a comprehensive range of tissues preserved. All tissues from these animals apart from the bronchi, salivary gland, sciatic nerve and tongue were examined microscopically. <u>Analysis of dose preparations</u> Analysis of the capsules containing Fipronil alone (used during the first 15 days of dosing) showed that slightly higher quantities than intended were present. Moreover the variation was degree of quite high. Therefore, the lactose admixture was introduced. Acceptable homogeneity of a lactose mixture containing 50 mg/g of Fipronil was confirmed (100.4% of nominal; coefficient of variation 5.15%). Moreover, stability of Fipronil in this carrier over 35 days storage was satisfactory (98.7%). The achieved concentrations of the test material were also acceptable (93 to 106% of nominal). Mortality One male given 2 mg/kg bw/d was killed in Week 11 following a severe reaction to treatment. Two males dosed with 5 mg/kg bw/d were killed (one in each of Weeks 31 and 34) after severe reactions to treatment. *Ante mortem* clinical signs for these animals included convulsive episodes, bodyweight loss, inappetence, and apparently impaired vision. Clinical signs Signs indicative of possible neurological disturbances occurred intermittently from Week 2 of treatment at both 2 and 5 mg/kg bw/d. They included convulsions, twitching or tremors of various muscle beds, nervous behaviour and abnormalities of gait and posture. All dogs given 5 mg/kg bw/d, and five males and three females dosed with 2 mg/kg bw/d were affected. Convulsions were observed in 1 male and 1 female at 2 mg/kg bw/d and in 2 males at 5 mg/kg bw/d. Other signs were seen at these dose levels included exaggerated rigidity or stiffness of the limbs, ataxia, muscular twitching and tremor, vocalisation, head nodding, behavioural changes in behaviour (aggression and nervousness) and activity patterns and resistance to dosing. There was no clear treatment-related effect at 0.2 mg/kg bw/d.	**x**

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**	
	Veterinary examinations A small number of dogs given either 2 or 5 mg/kg bw/d showed a number of treatment-related clinical signs including tense, nervous and excitable behaviour, abnormal stiffness or positioning of hindlimbs, facial twitching and hyperaesthesia. No treatment-related findings were seen at 0.2 mg/kg bw/d. *Ante mortem* examination of decedents confirmed their poor condition and indicated central nervous system impairment. Abnormalities included splayed limbs, abnormal stance and gait, twitching of the head muscles, and, in one male given 5 mg/kg bw/d, apparent visual impairment. Neurological examination (See Table A6.4.1.3-3) The treatment-related findings seen during the veterinary examinations were generally confirmed. Thus, a clear response to treatment was observed in three males and five females at the 5 mg/kg bw/d high dose level and in two females given 2 mg/kg bw/d. Findings comprised of tenseness, abnormalities of gait or stance (usually involving the hindlimbs) and exaggerated hopping and gag reflexes. Bodyweight (See Table A6.4.1.3-1) Bodyweight gain was particularly low in one female given 5 mg/kg bw/d over the first 26 weeks of treatment which affected the group mean bodyweight. Growth of other dogs in this group was similar to that of controls. However, the decedents exhibited weight loss prior to their termination. Food consumption (See Table A6.4.1.3-2) The food intake of treated animals was generally similar to that of the controls. Transitory periods of inappetence were seen in some dogs given 5 mg/kg bw/d which showed treatment-related clinical signs. Water was added to the diet to enhance palatability and, where necessary, a meat supplement provided. In addition, the daily ration was increased to 600 g to maintain consistency with the female given 0.2 mg/kg bw/d which was markedly overactive and losing weight. Ophthalmoscopy There were no treatment-related findings. Hematology No toxicologically significant findings were found in dogs surviving to study termination or in decedents. Clinical chemistry All changes were small, generally without trend and of a transitory nature. Therefore they were considered to be of no toxicological significance. Decedents given 5 mg/kg bw/d had slightly high plasma alanine and aspartate aminotransferase activities. The decedent dosed with 2 mg/kg bw/d exhibited marginally high plasma urea, total bilirubin and cholesterol. Urinalysis Composition of the urine was unaffected in both animals surviving to termination and in decedents.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (capsule study)**

	Organ weights There were no direct treatment-related effects on organ weights in animals killed after 52 weeks of treatment. Observed changes were attributed to lower bodyweight rather than a direct effect of treatment. Amongst decedents, one given 2 mg/kg bw/d and one dosed with 5 mg/kg bw/d had high spleen weights. Macroscopic pathology There were no treatment-related findings. Histopathology None of the histopathological findings was considered to be treatment-related.
5.3 Conclusion	Daily oral administration of 2 or 5 mg/kg bw/d of Fipronil to Beagle dogs for 52 weeks caused significant toxicity including mortality and clinical signs indicative of an effect on the central nervous system. However there were no associated histopathological changes. The No Observed Adverse Effect Level (NOAEL) was 0.2 mg/kg bw/d.
5.3.1 LO(A)EL	2.0 mg/kg bw/d
5.3.2 NO(A)EL	0.2 mg/kg bw/d
5.3.3 Other	
5.3.4 Reliability	1
5.3.5 Deficiencies	No

Table A6.4.1.3-1: Group mean bodyweight and bodyweight change (kg)

Week	Dose level (mg/kg bw/d)							
	Males				Females			
	0	0.2	2.0	5	0	0.2	2.0	5
	Bodyweight							
~~1~~ 0	8.3	8.3	8.3	8.4	7.5	7.3	7.4	7.3
13	11.3	11.2	11.5	11.2	9.8	9.5	9.6	9.3
26	12.7	12.8	12.9	12.8	11.0	10.5	~~10.5~~ 10.6	10.1
39	13.2	13.3	13.3	13.3	11.5	11.2	11.2	10.6
52	13.4	13.7	13.7	13.6	12.0	11.4	11.7	11.1
	Bodyweight Change							
0-13	3.0	2.9	3.1	2.8	2.4	2.3	2.2	2.1
13 – 26	1.4	1.6	1.4	1.6	1.1	1.0	1.1	0.8
26 – 39	0.5	0.4	0.4	0.5	0.5	0.7	0.5	0.5
39 52	0.2	0.5	0.4	~~0.5~~ 0.3	0.5	0.2	0.6	0.5
0 – 52	5.2	5.4	5.3	5.2	4.5	4.1	4.4	3.8

X

X

X

Table A6.4.1.3-2: Group mean food consumption (kg/animal/week)

Week	Dose level (mg/kg bw/d)							
	Males				Females			
	0	0.2	2.0	5	0	0.2	2.0	5
1-13	36.4	36.4	36.4	36.1	36.3	36.3	36.4	35.2
14-26	39.5	39.3	38.8	39.5	37.6	38.5	38.1	37.4
27-39	36.4	36.4	36.4	36.4	35.2	35.9	36.2	35.4
40-52	36.3	36.4	36.3	36.4	34.6	34.8	36.0	35.1
1-52	148.6	148.5	147.9	148.4	143.7	145.5	146.7	143.1

Table A6.4.1.3-3: Group incidence of findings at neurological examinations

Finding	Week	Dose level (mg/kg bw/d)							
		Males				Females			
		0	0.2	2	5	0	0.2	2	5
Tense	Pre treatment								
	13				3/6			1/6	3/6
	25	2/6	1/6	2/5	2/6		1/6	2/6	3/6
	39	2/6		3/5	2/4		1/6	2/6	2/6
	51			2/5	1/4	1/6	1/6	2/6	3/6
Stance – abnormal	Pre treatment								
	13		1/6						1/6
	25				2/6				2/6
	39				2/4				4/6
	51				1/4				4/6
Gait – abnormal	Pre treatment								
	13				1/6				
	25				1/6				
	39								
	51								
Hopping response – exaggerated	Pre treatment								
	13				1/6				
	25								2/6
	39				1/4				2/6
	51				1/4				
Gag reflex – exaggerated	Pre treatment						1/6		
	13								
	25								
	39		1/6						
	51						1/6		3/6

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: ~~*16 November 1992*~~ 2 November 1992 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.2 Purity: ~~*95.4%*~~ 96.8% 3.3.4.6 Controls: they received empty gelatin capsules during the first 15 days and thereafter received capsules containing lactose at a dosage of 100 mg/kg/day. 3.4.7 Clinical chemistry: *Parameters:* total cholesterol, creatine phosphokinase activity. 3.5.2 Gross and histopathology: ~~*Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, nerve, bone marrow, skin, and eyes.*~~Histopathology/microscopy: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes peripheral nerve, skin, eyes, femoral bone and articular surface, skeletal muscle, sciatic nerve, sternal bone and marrow, vagina, epididymides.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1.1 Clinical signs: *Signs indicative of possible neurological disturbance occurred intermittently from Week 2 in dogs s receiving* 2.0 or *5.0 mg/kg bw/d.* During a two-day period in week 34 of treatment marked signs of ill-health, which included a series of severe convulsions on one day, commencing approximately 2.5 hours after dosing, were noted for one male receiving 5.0 mg/kg/day. There was also some evidence of en apparent lack of vision. The dog was killed at the end of this period. *There was no clear effect of treatment in animals receiving 0.2 mg/kg bw/d. One female was markedly overactive* ~~*during Weeks 13 to 17*~~ during Weeks 13 to 18. *It developed lesions on the forepads and tail from continuous pacing around the pen* during Weeks 13 to 17. 4.3 Food consumption and compound intake: *was increased to* ~~*60 g per day*~~ 600 g per day. 4.5.1 Haematology: The packed cell volumes, haemoglobin concentrations and red blood cell counts recorded ante mortem for the two males receiving 5.0 mg/kg/day which were killed during the treatment period were slightly higher

	than their pretreatment values. However, they were generally similar to those recorded for treated and control animals at the investigations performed after 12 or 24 weeks and they were not, therefore, attributed to treatment.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Groups of 6 male and ~~5~~ 6 female pure-bred Beagle dogs* *Two males and two females from each group (only one male* and two females *from the* 2.0 mg/kg/day group ~~*highest dose level*~~ and only two females from the highest dose level*) were perfusion-fixed under anaesthesia*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.4.1/04** XXXX M&B 46030: Toxicity study by dietary administration to Beagle dogs for 52 weeks. XXXX (unpublished) (XXXX)	
1.2 Data protection	Yes/No	
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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes USEPA (=EPA) 82-1 (1984) JMAFF 59 NohSan no 4200 B.30 (1988) Also compliant with EU 88/302/EEC, B.30	
2.2 GLP	Yes	
2.3 Deviations	Extra specific neurological examinations and perfusion fixation for selected animals were also conducted	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	**X**
3.1.1 Lot/Batch number	PGS 963	
3.1.2 Specification	As given in Section 2	**X**
3.1.2.1 Description	Off-white powder	
3.1.2.2 Purity	95.4%	**X**
3.1.2.3 Stability	Stable	
3.2 Test Animals		
3.2.1 Species	Dog	
3.2.2 Strain	Pure bred Beagle	
3.2.3 Source	XXXX	
3.2.4 Sex	Male and female	
3.2.5 Age/weight at study initiation	Age: approx 21 to 24 weeks Weight: 9.4 – 11.6 kg (males) and 7.3– 10.0 kg (females)	**X**
3.2.6 Number of animals per group	10 – 5 males and 5 females	
3.2.7 Control animals	Yes	
3.3 Administration/ Exposure	Oral feed	
3.3.1 Duration of treatment	52 weeks	
3.3.2 Frequency of exposure	Daily	
3.3.3 Post exposure period	None	
3.3.4 **Oral**		
3.3.4.1 Type	food consumption per day ad libitum	**X**
3.3.4.2 Concentration	0.075, 0.3, 1 or 2/3 mg/kg bw/d	
3.3.4.3 Vehicle	None	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
3.3.4.4 Concentration in vehicle	n.a.	
3.3.4.5 Total volume applied	n.a.	
3.3.4.6 Controls	Blank diet	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: daily Each animal was subjected to veterinary examination before dosing commenced and after 11, 23, 35 and 47 weeks of treatment. Neurological examination: Before treatment started and after 12, 24, 37 and 50 weeks	
3.4.1.2 Mortality	Yes: daily	
3.4.2 Body weight	Yes: weekly	
3.4.3 Food consumption	Yes: daily	
3.4.4 Water consumption	Yes: daily	
3.4.5 Ophthalmoscopic examination	Yes: 12, 24 and 50 weeks	
3.4.6 Haematology	Yes Number of animals: all animals Time points: 12, 24 and 50 weeks Parameters: Packed cell volume, haemoglobin concentration, erythrocyte count, reticulocyte count, total and differential leukocyte count, blood film, platelet count, mean cell haemoglobin, mean cell volume, mean cell haemoglobin concentration, prothrombin time and thromboplastin time.	
3.4.7 Clinical chemistry	yes number of animals: all animals time points: 12, 24 and 50 weeks Parameters: sodium, potassium, chloride calcium and inorganic phosphorus, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, electrophoretic protein fractions, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase,	x
3.4.8 Urinalysis	Yes Number of animals: all animals Time points: 11, 23 and 49 weeks Parameters: appearance, volume,, specific gravity, pH, protein, glucose, blood, ketones, bilirubin, urobilingogen, nitrite	
3.4.9 Plasma analysis	After 1, 13, 24, 38 and 50 weeks blood samples were obtained from each dog. These samples were taken at the same time as those for haematology/blood chemistry. The samples from all animas were analysed for Fipronil and for the major rat metabolite XXXX	
3.5 Sacrifice and pathology		

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
3.5.1 Organ weights	Yes all dose groups organs: liver, kidneys, adrenals, testes, uterus with cervix, ovaries, spleen, brain, heart, lungs, pituitary, prostrate with urethra sample, thyroid with parathyroid, thymus	
3.5.2 Gross and histopathology	Yes all dose groups Macroscopic pathology: external features and orifices, neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, nerve, bone marrow, skin, eyes	
3.5.3 Other examinations		
3.5.4 Statistics	Standard deviations were calculated, as considered appropriate, using the sample statistic. The significance of inter-group differences in bodyweight gain, haematology, blood chemistry and urinalysis was assessed by Student's t-test using a pooled error variance. For organ weights, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher test was used to perform pairwise comparisons, otherwise a Dunnett's test was used. Inter group differences in macroscopic pathology and histopathology were assessed using Fisher's Exact test.	
3.6 Further remarks	None	
	4. RESULTS AND DISCUSSION	
4.1 Observations		
4.1.1 Clinical signs	<u>3 mg/kg bw/d:</u> Overall, neurological signs indicative of a response to treatment were noted in three males and two females of the high-dose group. One female later killed for humane reasons showed signs of neurological disturbance, including convulsive episodes, underactivity, prostration, slow respiration and body tremors. Neurological examination showed absence of visual placing reactions, depressed menace and startle reactions and abnormal gait. In the other 4 affected dogs, signs suggestive of neurological disturbance included convulsions and head nodding (one male), extensor rigidity of limbs (one male and one female) and twitching or tremors of various muscle beds (three males and one female). 1 mg/kg bw/d Signs of reaction to treatment were restricted to twitching of the whole body noted in Week 13 for one female and extensor rigidity of the limbs in Week 20 for another female. 0.3 and 0.075 mg/kg bw/d There were no findings that were considered treatment-related.	
4.1.2 Mortality	One female given 3.0 mg/kg bw/d was killed on Day 32 for humane reasons, after having displayed marked signs of ill-health and inappetence, suggestive of possible neurological disturbance. There were no other treatment-related deaths in other treatment groups.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
4.2	**Body weight gain**	There was no significant effect of treatment upon bodyweight gains. Over the first 26 weeks of treatment mean bodyweight gains were, when compared with controls, marginally low in males receiving 1 mg/kg bw/d and in males and females receiving 2 mg/kg bw/d. For males receiving 1 mg/kg bw/d this difference was attributed to the low values recorded for one animal. None of the individual bodyweight gains during the period were notably different from those observed in controls
4.3	**Food consumption and compound intake**	<u>Food consumption</u> Throughout the study the food intake of animals receiving Fipronil was generally unaffected by treatment, except that for the female sacrificed on Day 32.Periods of inappetence were noted from Week 23 to 35 and Weeks 44 to 52 for a female receiving 0.3 mg/kg bw/d and from Week 35 to 43 for a female receiving 0.075 mg/kg bw/d. Slight inappetence was also noted during Weeks 30 to 33 for a female receiving 1 mg/kg bw/d and during Weeks 50 to 52 for a female receiving 0.3 mg/kg bw/d. These periods generally correlated with signs of ill-health or signs indicative of disturbance of the oestrus cycle and were considered to have arisen fortuitously and to be unrelated to treatment <u>Test compound intake</u> The achieved dosages were very close to those intended throughout the treatment period. Overall dosages for treated groups were 0.074, 0.299, 0.998 and 1.992 mg/kg bw/d for males and 0.074, 0.295, 0.996 and 1.995 mg/kg bw/d for females receiving 0.075, 0.3, 1.0, and 2.0 mg/kg bw/d respectively. The achieved dosages for high dosage animals when given 3.0 mg/kg bw/d for the first 37 days and 2.0 mg/kg bw/d for the remaining five days of Week 6 were 2.840 and 2.822 mg/kg bw/d for males and females respectively.
4.4	**Ophthalmoscopic examination**	no effects
4.5	**Blood analysis**	
4.5.1	Haematology	Haematology investigations performed after 12, 24 and 50 weeks of treatment showed no changes considered to be related to treatment
4.5.2	Clinical chemistry	Blood chemistry investigations after 12, 24 and 50 weeks of treatment showed inter-group differences that essentially represented normal biological variation. None of these differences were attributed to treatment.
4.5.3	Urinalysis	No effects
4.6	**Sacrifice and pathology**	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
4.6.1 Organ weights	Analysis of organ weights for animals killed after 52 weeks of treatment showed no inter-group difference that could be attributed to treatment. When compared with control values, slightly higher absolute and bodyweight-relative spleen weights were noted in males given the high dosage. However in females given 0.3 mg/kg bw/d or more the opposite trend was apparent. These variations in spleen weight were considered fortuitous	
4.6.2 Gross and histopathology	Macropathological examination of animals killed after 52 weeks showed a few abnormalities that were considered to represent the expected findings in Beagle dogs of this age. None of these changes were attributed to treatment. The incidence of swollen or large spleens of males given the high dosage was slightly higher than that of their controls. However, no similar difference was noted in other treated male groups or in treated females and this was not considered to be attributable to treatment. There were no histopathological findings considered to be related to treatment in animals killed after 52 weeks. The incidence of hyperplasia of the red pulp in the spleen in males given the high dosage was slightly higher than that of the controls, although within the expected range. This difference was considered to probably reflect the degree of exsanguinations of individual animals rather than representing an effect of treatment.	
4.7 Other	**Veterinary examination**: Routine veterinary examinations performed after 11, 23, 35 and 47 weeks did not reveal any findings that were related to treatment.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
	Neurological examination: Neurological examinations during the course of this study showed exaggeration of the flexor response after 12, 24 and 50 weeks in one male receiving 2.0 mg/kg bw/d. Among animals receiving 0.3 mg/kg bw/d, this finding was noted in one male after 24 weeks and in a further male after 37 and 50 weeks. The effects seen in the animals receiving 0.3 mg/kg bw/d were not attributed to treatment because of the lack of dosage-relationship and absence of any other signs of reaction to treatment at this dose. The relationship to treatment of this finding in one male receiving 2 mg/kg bw/d is considered to be equivocal. Isolated incidences of exaggeration of the tonic neck reflex were also noted in two males, in Week 13 for an animal receiving 2 mg/kg bw/d and in Week 51 for another animal receiving 0.075 mg/kg bw/d. However, because of the isolated nature of these findings and the clear absence of dosage-relationship they could not be confidently ascribed to treatment. A number of other abnormalities were noted for treated dogs. However, these were either also apparent before commencement of treatment or occurred at a similar incidence in the control animals and were not, therefore, ascribed to treatment. **Plasma analysis** Analysis of plasma obtained after 1, 13,24, 38 and 50 weeks showed the presence of Fipronil and a major metabolite, XXXX, for animals of all treated groups. Plasma levels showed a clear dosage-relationship and the concentration of M&B 46136 was higher at all occasions than that of the parent Fipronil. There was no evidence of any significant sex difference in plasma levels and no accumulation of either the parent material or the metabolite was noted as the study progressed.	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Groups of 5 male and 5 female pure-bred Beagle dogs were given dietary dose levels of 0, 0.075, 0.3, 1 or 3/2 mg/kg bw/d of Fipronil for 52 consecutive weeks. The highest dose level, 3 mg/kg bw/d, was reduced to 2 mg/kg bw/d after 38 days of administration because of significant toxicity. The dogs were about 21 to 24 weeks old at the start of treatment and weighed between 9.4 kg and 11.6 kg (males) and 7.3 kg and 10.0 kg (females). They were housed individually and were acclimatised for 4 weeks. Test diets were prepared at weekly intervals and stored at room temperature. The concentration was adjusted for each group and sex at weekly intervals over the first four weeks and then at 4-weekly intervals to provide the required dosages. Prior to administration of the diets, water (about 30% by weight of dry diet) was added to reduce the amount of airborne dust and enhance palatability. Diets were offered to the dogs twice daily throughout the study for at least 3.5 hours.	x

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
	Homogeneity of mixing Fipronil with the diet and its stability in this medium had been established in a previous study. In the present study, the achieved concentration of the test material was checked in diets prepared for Weeks 1-4, 12, 20, 28, 36, 44 and 52. Animals were observed daily for mortality and clinical signs. A veterinary examination was carried our on each animal before the start of treatment and after 11, 23, 35 and 47 weeks of treatment. In addition, a neurological examination was performed on each dog prior to the start of dosing and after 12, 24, 37 and 50 weeks. This comprised examining reflexes, reactions and general observations (cranial reflexes: direct and indirect pupillary light, palpebral, gag, menace and general head examinations for cranial nerve response; segmental reflexes: flexor, patellar, crossed extensor, cutaneous trunci; postural reactions: placing, righting, hopping, extensor postural thrust and tonic neck; general responses: abnormalities of gait and stance, behavioural changes and tremors or other dyskinesias). Individual bodyweights were recorded at weekly intervals and prior to necropsy. Individual food consumption was recorded daily and achieved dosages were calculated weekly. An ophthalmic examination was conducted prior to the start of dosing and after 12, 24 and 50 weeks of treatment. Blood samples for hematology and clinical chemistry analyses were taken at these time points. Urine samples were collected for analysis before treatment started and after 11, 23 and 49 weeks. Plasma samples obtained prior to treatment and after 1, 13, 24, 38 and 50 weeks were analysed for Fipronil and the major metabolite, XXXX. Blood samples were also taken from each surviving dog after 50 weeks of treatment and the serum stored frozen. All animals were necropsied and examined externally and internally (cranial, thoracic, abdominal and pelvic cavities and their viscera) for macroscopic changes. Selected organs were weighed and a wide range of tissues preserved and examined microscopically.	
5.2 Results and discussion	<u>Analysis of diet preparations</u> Acceptable overall mean achieved concentrations of Fipronil in dry diet (checked prior to use) were obtained. Values were 91.9 + 4.9%, 93.1 + 4.1%, 95.5 + 4.7% and 95.9 + 8.3% for males and 92.4 + 4.3%, 95.5 + 2.7%, 94.1 + 8.7% and 96.7 + 5.5% for females at 0.075, 0.3, 1 and 3/2 mg/kg bw/d, respectively. Achieved test material intakes The achieved dose levels were very close to those intended throughout the treatment period. Overall mean intakes were 0.074, 0.299, 0.998 and 1.992 mg/kg bw/d for males and 0.074, 0.295, 0.996 and 1.995 mg/kg bw/d for females at 0.075, 0.3, 1 and 2 mg/kg bw/d, respectively. Achieved intakes at the initial highest dose level, 3 mg/kg bw/d, over the first 37 days and at 2 mg/kg bw/d for the remaining five days of Week 6 were 2.840 and 2.822 mg/kg bw/d for males and	**x**

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
	females, respectively. <u>Mortality</u> One female given 3 mg/kg bw/d was killed on Day 32 following a severe reaction to treatment. Tremors and underactivity were noted on Day 10 followed by a convulsive episode on Day 11 approximately 18 hours after the second feed of the previous day. Further convulsions underactivity, prostration and slow respiration were also seen. Severe signs were still apparent on Day 32. The animal's poor condition was confirmed at a veterinary examination and at necropsy. Neurological examination at necropsy revealed a range of abnormalities including absence of visual placing reactions, depressed startle reactions and moderately abnormal gait. Blood samples showed high packed cell volume, hemoglobin concentration and erythrocyte count as well as slightly high alkaline phosphatase activity, high total plasma cholesterol and protein concentrations compared with pre-treatment values. Although liver weight was slightly high, there were no histopathological findings.	

Section A6.4.1 **Annex Point IIA, VI.6.4**	**52-week oral toxicity test in dogs (dietary study)**	
	Clinical signs In addition to the signs seen in the decedent female at 3/2 mg/kg bw/d, signs indicative of neurological disturbances (convulsions, head nodding, extensor rigidity of limbs and twitching or tremors of various muscles) were seen in three males and one female in this dose group and in two females given 1 mg/kg bw/d. Convulsive episodes were observed on Day 36 in one male at 3 mg/kg bw/d and in Week 45 for the same individual which was then receiving 2 mg/kg bw/d. It also displayed abnormal head nodding during Weeks 5 and 6. Exaggerated rigidity of limbs occurred during Weeks 20 to 25 in one male given 2 mg/kg bw/d, in Weeks 20 to 41 in one female at this same dose level and in Week 20 in one female being dosed with 1 mg/kg bw/d. Twitching or tremors of various muscle beds were occasionally seen in a few high dose level dogs, mainly in head muscles, but sometimes a more general response was noted. There were no treatment-related clinical signs at 0.075 and 0.3 mg/kg bw/d. Bodyweight There was no significant effect of treatment upon bodyweight gains. Food consumption Food intake was unaffected by treatment. Veterinary examination There were no treatment-related findings. Neurological examination Neurological examinations during the course of the study showed exaggeration of the flexor (withdrawal) response after 12, 24 and 50 weeks in one male fed 2 mg/kg bw/d. There were no other treatment-related neurological findings. Ophthalmoscopy No treatment-related findings were observed. Hematology There were no changes considered to be related to treatment. Clinical chemistry Clinical chemistry parameters were unaffected. Urinalysis No treatment-related effects were seen. Organ-weights, gross and histpathology No treatment-related effects were seen.	**x**
5.3 Conclusion	Dietary administration of 3 mg/kg bw/d of Fipronil caused significant toxicity including mortality and clinical sings indicative of a central nervous system effect. Neurological signs continued when the dose level was reduced to 2 mg/kg bw/d. However, there was no associated histopathological change. Treatment with 1 mg/kg bw/d also induced clinical signs indicative of neurological involvement. The NOEL was 0.3 mg/kg bw/d.	
5.3.1 LO(A)EL	1.0 mg/kg bw/d	
5.3.2 NO(A)EL	0.3 mg/kg bw/d	

Section A6.4.1 52-week oral toxicity test in dogs (dietary study) **Annex Point IIA, VI.6.4**		

5.3.3 Other		
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.2 Purity: ~~*95.4%*~~ 96.8% 3.2.5 Age/weight at study initiation/5.1 Materials and methods : *Weight~~: 9.4~~ 8.9 – 11.6 kg (males)* 3.3.4.1 Type: Weighed amounts of diet (200g/dog) were provided twice each day. 3.4.7 Clinical chemistry: *Parameters:* creatine phosphokinase activity, triiodithyronine (T3), thyroxine (T4) 3.5.2 Gross and histopathology: ~~*Microscopic pathology: brain, spinal cord, thyroid, parathyroid, liver, kidneys, sciatic nerve, nerve, bone marrow, skin, and eyes.*~~Histopathology/microscopy: brain, spinal cord, pituitary, thyroid, parathyroid, thymus, oesophagus, salivary glands, stomach, small and large intestines, liver, pancreas, kidneys, adrenals, spleen, heart, trachea, lungs, aorta, gonads, uterus, female mammary gland, prostate, urinary bladder, lymph nodes peripheral nerve, skin, eyes, femoral bone and articular surface, skeletal muscle, sciatic nerve, sternal bone and marrow, vagina, epididymides.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *Values were 91.9 ~~+~~ ± 4.9%, 93.1 ~~+~~ ± 4.1%, 95.5 ~~+~~ ± 4.7% and 95.9 ~~+~~ ± 8.3% for males and 92.4 ~~+~~ ± 4.3%, 95.5 ~~+~~ ± 2.7%, 94.1 ~~+~~ ± 8.7% and 96.7 ~~+~~ ± 5.5% for females* *Neurological examination:* Among animals receiving 0.3 mg/kg bw/d, this finding was noted in one male after 24 weeks and in a further male after 37 and 50 weeks. The effects seen in the animals receiving 0.3 mg/kg bw/d were not attributed to treatment because of the lack of dosage-relationship and absence of any other signs of reaction to treatment at this dose. The relationship to treatment of this finding in one male receiving 2 mg/kg bw/d is considered to be equivocal. Isolated incidences of exaggeration of the tonic neck reflex were also noted in two males, in Week 13 for an animal receiving 2 mg/kg bw/d and in Week 51 for another animal receiving 0.075 mg/kg bw/d. However, because of the isolated nature of these findings and the clear absence of dosage-relationship they could not be confidently ascribed to treatment.
Reliability	1
Acceptability	acceptable
Remarks	

COMMENTS FROM …
Date
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Section A6.4.2 Subchronic dermal toxicity test **Annex Point IIA, VI.6.4**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Available dermal studies (acute dermal toxicity in rats and rabbits, and a 21-day dermal toxicity study in rabbits; see sections A6.1.2 and A6.3.2) have demonstrated that Fipronil exerts substantially lower toxicity after acute or repeated dermal compared to oral exposure. The conduct of a percutaneous 90-day toxicity study in the rat is not considered to be required since route-to-route extrapolation is not restricted in any way.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Results and discussion	Agree with applicant's version.
Materials and methods	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	One death was observed, enlargement of the liver, thyroid follicular hypertrophy and modifications of the clinical chemistry (urea, total protein, albumin)… were noted by repeated oral dose; not by repeated dermal dose.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.4.3 Subchronic inhalation toxicity test **Annex Point IIA, VI.6.4**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	(1) The vapour pressure of Fipronil (IIA, 3.2) was determined experimentally under GLP to a value below 3.7 x 10^{-9} hPa (25°C). Therefore, Fipronil is not a volatile substance. (2) In consideration of the intended uses, inhalation is considered to be a negligible route of exposure. In conclusion, the performance of a subchronic inhalation toxicity study in the rat is consequently not considered to be required predominantly for lack of exposure.	
Undertaking of intended data submission [ ]		

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

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A6.5/01** 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. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes USEPA (=EPA) 83-5 (1984) Also complies with EU 88/302/EEC B33(1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	A satellite group was included which was maintained on untreated diet for 13 weeks after completion of 52 weeks of treatment to assess the reversibility of any findings. Thyroid hormones and pituitary thyroid stimulating hormone levels were measured	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Slightly yellow powder	**X**
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	CD	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	Age: 35 to 42 days Weight 189 to 192 g (males) and 158 to 162 g (females)	
3.2.6	Number of animals per group	100 (50 male and 50 female) plus an additional 30 (15 male and 15 female) for evaluation of chronic toxicity and an additional 30 (15 male and 15 female) to assess reversibility	
3.2.7	Control animals	No	**X**
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	104 weeks	**X**
3.3.2	Frequency of exposure	Continuous	
3.3.3	Post exposure period	None or 13 weeks	

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
3.3.4 **Oral**		
3.3.4.1 Type	In food	
3.3.4.2 Concentration	0.5, 1.5, 30, 300 mg Fipronil/kg feed (ppm)	
3.3.4.3 Vehicle	Powdered rodent diet (Laboratory Animal Diet No.2, source: Special Diets Services, Witham, Essex, England)	
3.3.4.4 Concentration in vehicle	See 3.3.4.2	
3.3.4.5 Total volume applied	Food consumption: ad libitum	
3.3.4.6 Controls	Vehicle (Plain diet)	
3.4 Examinations		
3.4.1 Observations		
3.4.1.1 Clinical signs	Yes: twice daily	
3.4.1.2 Mortality	Yes: twice daily	
3.4.2 Body weight	Yes: weekly for 14 weeks and then fortnightly	
3.4.3 Food consumption	Yes: weekly	
3.4.4 Water consumption	Yes	**x**
3.4.5 Ophthalmoscopic examination	Yes: prior to start of treatment (all animals) after 50 weeks and after 13 weeks without substance administration following 50-wk treatment ("reversibility" controls and high dose group only)	**x**
3.4.6 Haematology	Yes Number of animals: 20 (10♂ and 10♀) from each dosing group Time points: 24, 50, 76, 88(♂) and 90(♀) weeks and after 12 weeks of reversibility following 52-week treatment Parameters: Packed cell volume, haemoglobin concentration, erythrocyte count, total and differential leucocyte count, platelet count Blood film, reticulocyte count, mean cell haemoglobin concentration, mean cell haemoglobin, mean cell volume, prothrombin time	**x**
3.4.7 Clinical chemistry	Yes Number of animals: 20 (10♂ and 10♀) from each dosing group Time points: 24, 50, 76, 88(♂) and 90(♀) weeks and after 12 weeks of reversibility following 52-week treatment alkaline phosphatase activity, alanine amino-transferase activity, aspartate amino-transferase activity, creatin phosphokinase activity, urea concentration, creatinine concentration, glucose concentration, total bilirubin concentration, total cholesterol concentration, total protein concentration, electrophoretic protein fractions, sodium, potassium chloride, calcium and inorganic phosphorus concentration.	**x**
3.4.8 Urinalysis	Yes Number of animals: 20 (10♂ and 10♀) from each dose group Time points: 23, 49, 75, 87(♂) and 90(♀) weeks and after 6 and 11 weeks off-treatment following 52-week treatment ("reversibility") Parameters: appearance, volume, specific gravity, pH, protein, glucose, blood, total reducing substances, ketones, bilirubin, urobilinogen, nitrite.	**x**
3.5 Sacrifice and pathology		
3.5.1 Organ weights	Yes All animals Organs: Adrenals, brain, heart, kidneys, liver, lungs with mainstem bronchi, ovaries, pituitary, prostate, spleen, testes, thymus, thyroid with parathyroids, uterus with cervix.	

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
3.5.2 Gross and histopathology	Yes All dose groups/high dose group and controls, other dose groups only if effects Organs: adrenals, aorta, brain caecum, colon, duodenum, epididymides, eye and optic nerve, femoral bone and marrow, heart, ileum, jejumum, kidneys, liver, lungs with mainstem brochi, lymph nodes, mammary gland oesophagus, ovaries, pancreas, pituitary prostate, rectum, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord, spleen, sternum, stomach, testes, thymus thyroid with parathyroid, trachea, urinary bladder, uterus with cervix, vagina	x
3.5.3 Other examinations		
3.5.4 Statistics	Inter-group differences in mortality were analysed by Cox's proportional hazards model and Tarone's partition of the Chi-square statistic. The significance of inter-group differences in haematology and urinalysis was assessed by Student's t-test. Pairwise Mann-Witney U tests were applied when the variance of one or more groups was zero. For organ weights and bodyweight changes, homogeneity of variance was tested using Bartlett's test. Whenever this was found to be statistically significant a Behrens-Fisher or Dunnett's test was used. Fisher's Exact test was applied where appropriate to the distribution of macroscopic or microscopic entities.	
3.6 Further remarks	**4. RESULTS AND DISCUSSION**	
4.1 Observations 4.1.1 Clinical signs	(See Table A6.5-1) Convulsive episodes, lasting up to 25 minutes, were noted for eight males and twelve females receiving 300 ppm, one male and three females receiving 30 ppm and three males receiving 1.5 ppm. In 6 males and 1 female exposed to 300 ppm, and in 1 female of the 30 ppm group, a single convulsive episode was noted (only) during weeks 1-3 of treatment. Thereafter, further convulsive episodes were seen again in other treatment-group animals after 21 weeks of continued exposure. At 1.5 ppm, one male rat with a convulsion was seen during week 23, another male during weeks 69 and 71, and the third male during week 61. Four males and three females receiving 300 ppm, one female receiving 30 ppm and one male receiving 1.5 ppm died following convulsive episodes. Irritability, overactivity, vocalisation, salivation and aggressive behaviour were noted throughout the treatment period among females receiving 300 ppm and, to a lesser degree, females receiving 1.5 or 30 ppm. Among males receiving 300 ppm the number of thin animals was higher than among controls and the number of obese animals lower; this reflected the effect on bodyweight. Other signs observed during the treatment period were those expected in CD rat at this laboratory and were not related to treatment. The neurological changes observed during the treatment period were not evident during the reversibility period.	x

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
4.1.2 Mortality	During the early part of the study the number of animals that died or were killed was slightly higher for the groups receiving 300 ppm than for the controls. This difference was considered to reflect the number of animals that died after convulsive episodes during the first few weeks of treatment. The number of deaths among other treated groups was similar to that of their respective controls during this period. In the second half of the study, as overall mortality increased, the number of deaths among females receiving 30 ppm was greater than among their controls. However, no similar effect was noted among females receiving 200 ppm and the observation is therefore considered to have arisen by chance. The group-distribution of deaths during the reversibility period was not related to previous treatment.	x
4.2 Body weight gain	(See Table A6.5-2) The bodyweight gains of males and females receiving 300 ppm were markedly lower than those of their controls during the first week of treatment (42 and 46% of the controls for males and females respectively) and continued to be significantly lower than controls thereafter. After 52 weeks of treatment the gains were 85 and 82% of the controls for males and females, respectively. The overall weight gains of males receiving 30 ppm were slightly lower than control values throughout the treatment period; this difference was not statistically significant. Overall bodyweight gains of females receiving this dietary concentration were similar to those of the controls during the first 52 weeks of treatment but declined sharply thereafter resulting in a difference at the end of treatment, which was statistically significant. The decline may be associated with the high mortality noted for this group during this period. There was no effect of treatment of the bodyweight gains of males and females receiving 0.5 or 1.5 ppm There was no clear improvement in weight gain, relative to the controls, among animals, receiving 30 or 300 ppm following cessation of treatment during the reversibility period.	x
4.3 Food consumption and compound intake	(See Tables A6.5-3 and 4) Males and females receiving 300 ppm consumed less food than their respective controls during the first week of treatment; in the males this persisted into the second week of treatment. Total food intakes were, however, broadly similar to controls for all treated groups. The 13-week respite from treatment did not have any clear effect upon the quantity of food consumed by previously treated groups. The efficiency of food conversion of animals receiving 300 ppm was markedly lower than that of their respective controls during the first week of treatment, but similar thereafter. The efficiency of food conversion of other treated groups was similar to that of the controls over the first 14 weeks of treatment. The overall achieved dosages, calculated for weeks 1 to 90, for animals receiving 0.5, 1.5, 30 or 300 ppm were, for males approximately 0.02, 0.06, 1.3, and 13 mg/kg bw/d and, for females 0.03, 0.08, 1.6, and 17 mg/kg bw/d.	x
4.4 Ophthalmoscopic examination	Ophthalmoscopic examination after 50, 87(♂)/90(♀) weeks of treatment of controls and animals receiving 300 ppm did not reveal any treatment-related findings.	
4.5 Blood analysis		

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
4.5.1 Haematology	(See Table A6.5-5) Animals receiving 300 ppm generally had lower packed cell volumes, haemoglobin concentrations, and erythrocyte counts than controls and this was occasionally associated with low mean cell haemoglobin and mean cell volumes. Animals receiving 30 ppm were often similarly affected and there were occasions when those treated at 1.5 ppm also showed the same trend. There was no evidence for persistence of this effect after 12 weeks of the reversibility period Prothrombin times were generally slightly shorter than those of controls in rats treated at 300 ppm. Females treated at 30 ppm were also apparently affected on some occasions. Although the prothrombin times of females treated at 30 or 300 ppm remained lower than those of controls after 12 weeks of the reversibility period it is noted that there were no marked inter-group differences after 88 or 90 weeks of treatment. A trend towards higher platelet counts than in controls was noted after 76 and 88 or 90 weeks of treatment in animals treated at 300 ppm and males receiving 30 ppm. Other inter-group differences were occasionally noted at routine examinations but these were inconsistent between examinations, occurred in one sex only or were without trend or dosage-relationship and are not considered to be related to treatment. Haematology findings from animals killed during the treatment period generally reflected their poor health status – there was no clear evidence of ay treatment related trends among these animals. Examination of blood smears taken after 50, 76 and 88/90 weeks of treatment did not reveal nay treatment-related abnormalities.	X
4.5.2 Clinical chemistry	(See Table A6.5-6) Changes of possible toxicological significance comprised high plasma cholesterol concentrations, particularly in females, high calcium concentrations, high total protein concentrations, low albumin and high alpha- and beta globulin concentrations and low albumin to globulin ratios. All of these changes were apparent on most occasions of examination in animals treated at 300 ppm. Among animals receiving 30 ppm, plasma cholesterol levels were occasionally affected and calcium concentrations were only affected after 24 weeks of treatment. There was evidence in these animals, throughout the treatment period, of a similar effect on plasma proteins to that seen at 300 ppm. After 76 and 88 weeks of treatment at 1.5 ppm there were indications of a disturbance in plasma protein concentrations similar to that seen at the higher treatment levels. Although after 76 weeks of treatment males receiving 0.5 ppm presented a similar variation in plasma protein concentrations, this was not seen on any other occasion in these animals nor in females and cannot be confidently ascribed to treatment. There was not other evidence of an effect in this group. After 12 weeks of the reversibility period high cholesterol and calcium concentrations together with high alpha and beta-globulins and associated high total protein and low albumin to globulin ratios were still apparent in females at had had previously received 300 ppm. There were no similar findings at lower dietary concentrations nor in males that had previously received 300 ppm.	**X**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	A number of other apparently treatment-related changes were noted: these included low alkaline phosphatase activities in females, generally slightly low amino-transferase activities, low bilirubin concentrations in males, high urea concentrations, in females after 76 and 90 weeks and various changes in plasma electrolyte concentrations, however, none of these effects were of toxicological significance and they could not, with confidence, be ascribed to treatment. Biochemical findings from animals killed during the treatment period generally reflected their poor state of health – there were no consistent findings in treated animals compared to the controls that could be confidently attributed to treatment.	
4.5.3 Urinalysis	(See Table A6.5-8) The urinary pH of animals receiving 300 ppm was slightly lower than that of the controls throughout the treatment period, with the exception of males on the first sampling occasion. Low pH was also noted in the second half of the study for animals receiving 30 ppm. On most occasions, particularly early in the treatment period, protein concentrations tended to be higher than control values for males and females receiving 300 ppm. A similar trend towards higher protein concentrations was also noted for males receiving 30 ppm. High urinary volumes associated with low specific gravities, were noted after 87 weeks in males receiving 30 or 300 ppm. After 6 and 11 weeks of the respite period low urinary pH was still evident in males that had received 30 ppm and in males and females that had received 300 ppm. More animals which had received 30 or 300 ppm had higher urinary protein concentrations that the controls	**X**
4.6 Sacrifice and pathology		
4.6.1 Organ weights	(See Table A6.5-9) Among animals killed for interim or terminal examinations absolute and bodyweight-relative liver and thyroid weights were significantly higher than control values fro males and females receiving 300 ppm. The absolute liver weights were generally higher than in the controls fro animals receiving 30 ppm but the differences did not always attain statistical significance; bodyweight relative liver weights were significantly high for these animals.	**X**

Section A6.5 **Annex Point IIA, VI.6.5**		**Chronic toxicity and carcinogenicity study in rats**	
		The absolute and bodyweight-relative kidney weights of animals receiving 30 or 300 ppm killed for terminal examination after 89 or 91 weeks were higher than control values, as were the adrenal weights of males receiving these concentrations. The absolute and bodyweight-relative spleen weights of males receiving 300 ppm wee also high compared with controls. Statistical significance was not attained in all cases. After 13 weeks of respite from treatment, higher bodyweight-relative kidney and liver weights, when compared with the controls, were recorded among males and females that had received 300 ppm. The absolute weights of these organs were similar to, or higher than, the controls, despite the bodyweight deficit, although none of the differences attained statistical significance. Other differences from control values, which occasionally attained statistical significance, were considered to reflect the inter-group disparity in bodyweight. Examination of the organ weights of animals killed or dying during the treatment period indicated that the weights of the livers and thyroids of males and females that received 300 ppm and the kidneys of males receiving 300 ppm tended to be higher than those of controls which died or were killed prematurely.	
4.6.2	Gross and histopathology	**Macropathology** (see Table A6.5-10)**:** Higher incidences, compared with controls, of large and/or pale kidneys and large livers, adrenals and thyroids were noted for males receiving 300 ppm which died or were killed during the study. At macroscopic examination of animals killed for interim examination after 52 weeks of treatment, large livers were seen in four males and one female that received 300 ppm, and enlarged thyroids were seen in one male that received 30 ppm and in one male that received 300 ppm. There were no other macroscopic abnormalities noted at interim examination that could be confidently attributed to treatment. At examination after the respite period there were no macroscopic findings which could definitely be ascribed to previous treatment with Fipronil although large kidneys were noted in occasional males which had received 30 or 300 ppm and large livers in two males which had received 300 ppm Among animals killed for terminal examination after 89 or 91 weeks the incidences of large and pale kidneys were higher than controls fro males and females that received 30 or 300 ppm. The incidences of granular kidneys, large livers and large thyroids were also higher than controls for males which received 300 ppm; slightly high incidences of these findings were also noted for females which received 300 ppm and fro males which received 30 ppm Other findings were those expected in animals of this age and strain at this laboratory	**x**
		Micropathology (see Tables A6.5-11, -12 and -13)**:** Findings considered to be of toxicological significance were noted in the kidneys of animals from all phases. Progressive senile nephropathy was noted in animals from all groups although the incidence and severity of the findings was greater among Toxicity Phase males and females which received 300 ppm and among Oncogenicity Phase males and females which received 30 or 300 ppm.	**x**

Section A6.5 **Annex Point IIA, VI.6.5**		**Chronic toxicity and carcinogenicity study in rats**	
		The effect was most marked in the males. ; A linear-by-linear association test indicated a positive association between dietary concentrations and severity among Toxicity Phase males and Oncogenicity Phase males and females. This renal change was still apparent after treatment was discontinued for a period of 13 weeks; the incidence of nephropathy was higher than controls among females which had received 30 or 300 ppm and the severity was greater among animals which had received 1.5 ppm or more than among controls. A linear-by-linear association test indicated a positive association between severity and the dietary concentration previously received for both males or females. Other findings were of types and incidences commonly seen in rats of this age and strain at these laboratories. Neoplastic findings considered to be related to treatment were recorded in the thyroid gland. When all animal assigned to the Oncogenicity Phase were considered together, irrespective of time of death, significantly higher incidences than in the controls of benign follicular cell adenomas were noted for males and females which received 300 ppm and of malignant follicular cell carcinomas for males which received 300 ppm. Although higher than control incidences of follicular cell tumours were also noted for males which received 1.5 or 30 ppm the apparently high incidence in these animals were well within the expected background range from concurrent studies in the performing laboratory and reflect an abnormally low incidence in the controls. These findings were apparent in animals which died or were killed during treatment as well as those killed for terminal examination. Among animals assigned to the Toxicity Phase there were no tumours considered to be related to treatment. Six Reversibility Phase animals had thyroid follicular cell tumours. One Oncogenicity Phase male that received 300 ppm had a rare tumour, a malignant chardoma. This tumour, though rare, has previously been diagnosed and is not considered to be related to treatment. Other types of tumours were of types commonly seen in rats at this laboratory and occurred with the expected frequencies.	**x**
4.7	**Other**	**Thyroid hormones** (see Table A6.5-7) : The concentration of triiodothyronine (T_3) in the serum of males receiving 300 ppm was slightly lower than that of the controls after the first week of treatment; no differences were apparent at subsequent examinations during the treatment period. Following the cessation of treatment however, the serum concentration of T_3 was higher than controls after 4, 7 and 11 weeks of reversibility among females that had received 300 ppm and after 7 and 11 weeks among females that had received 30 ppm. Circulating thyroxine (T_4) levels were consistently below control values for treatment group males at and above 1.5 ppm, in a dose-related manner.	**x**
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	For the assessment of oncogenic potential, groups of 50 male and 50 female Sprague Dawley CD rats were given dietary concentrations of 0, 0.5, 1.5, 30 or 300 ppm of Fipronil for 89 weeks. The duration was intended to be 104 weeks but was shortened to 89 weeks for males and	**x**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	91 weeks for females because of poor survival. At the start of treatment, the animals were 35 to 42 days of age and the group mean body weights were 189 to 192 g (males) and 158 to 162 g (females). They were acclimatised for 14 days prior to the start of treatment and were housed by sex in groups of 5. For the evaluation of chronic toxicity, further groups of 15 male and 15 female Sprague Dawley CD rats were fed the same dietary concentrations for 52 weeks. Additional groups of 15 males and 15 females, assigned to each group were fed untreated diet for a further 13 weeks on completion of the 52-week treatment period to assess the reversibility of any treatment-related findings. Batches of test diets were prepared at weekly intervals by directly mixing the test material with the diet. Homogeneity of mixing 400 ppm of Fipronil with the diet, and the stability of the test material in this medium following 21 days storage at room temperature at 300 ppm was confirmed in previous studies. For the present study, homogeneity of mixing and stability at 0.5 and 1.5 ppm was assessed prior to the start of treatment. Test diet samples from Weeks 1-4, 12, 20, 28, 36, 44, 52, 60, 68, 76, 84 and 92 were analysed for the achieved concentration of Fipronil. Animals were observed at least twice daily for mortality and clinical signs. A detailed weekly examination, including palpation, was also performed. Individual bodyweights were recorded weekly for the first 14 weeks of treatment, fortnightly thereafter and at necropsy. Food consumption for each cage group was recorded weekly throughout. Water intake was checked by visual inspection of the water bottles. An ophthalmic examination was conducted on all rats prior to the start of treatment, on all surviving animals from the control and highest dose level from the chronic toxicity phase after 50 weeks of treatment and at the end of the 13-week reversibility phase. In addition, the eyes of surviving rats from the control and highest dose oncogenicity groups were examined after 87 weeks (males) and 90 weeks (females). Blood samples for hematology and clinical chemistry from the 10 male and 10 female rats from each group were taken after 24 and 50 weeks of treatment (chronic toxicity phase) and after 76 and 88 weeks (males only) and 90 weeks (females only) from the oncogenicity phase. All samples were collected after overnight fasting. Additionally, after 1, 4, 12, 24 and 50 weeks and after 2, 4, 7 and 11 weeks of the 'off-dose' reversibility period, blood samples were taken from the ten male and ten female rats in each group for measurement of triiodothyronine (T_3), thyroxine (T_4) and thyroid stimulating hormone (TSH) concentrations. Urinalysis was performed on ten males and ten females per group after 23 and 49 weeks of treatment (Chronic toxicity phase) and after 75 and 87 weeks (males only) and 90 weeks (females only) weeks of treatment (Oncogenicity phase). In addition, urinalysis was performed on a similar number of animals from the Reversibility phase after 6 and 11 weeks. At necropsy, all animals were examined externally and internally (cranial, thoracic, abdominal and pelvic cavities and their contents) for macroscopic changes. Selected organs were weighed and a comprehensive range of tissues preserved. All tissues (except the right eye and optic nerve, harderian glands, cranial mammary gland,	**x**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
5.2 Results and discussion	right submandibular salivary gland, right sciatic nerve and tongue) were examined microscopically from the control and highest dose groups of the chronic Toxicity and oncogenicity phases and from all decedents. The liver, kidneys, lungs and thyroid from all other dose levels of these two main phases and from all rats of the Reversibility phase were also examined. All abnormalities were evaluated microscopically. <u>Analysis of diet preparations</u> At 0.5 and 1.5 ppm of Fipronil, acceptable homogeneity in the diet was demonstrated since values of 98.8% and 107% (coefficients of variation 6.4 and 4.51%), respectively, were achieved. Satisfactory stability was also shown at these concentrations with values of 101% and 102% of nominal obtained at 0.5 and 1.5 ppm, respectively, following 14 days of storage at room temperature. The concentration of Fipronil in diet preparations used in Weeks 1-4 and at eight-week intervals thereafter averaged 104+12%, 102+13.5%, 95.7+4.2% and 98.4+3.4% of nominal at 0.5, 1.5, 30 and 300 ppm, respectively. The achieved concentrations were within +15% of nominal. <u>Mortality</u> At 300 ppm, the number of animals that died or were killed during the early part of the study was slightly higher than in the controls. This difference reflected the number that died after convulsive episodes during the first few weeks of treatment (see Clinical signs below). The number of deaths among other treated groups during this period was similar to the controls. Mortality during the later treatment period was similar in all groups. <u>Clinical signs</u> (See Table A6.5-1) Convulsive episodes, lasting up to 25 minutes, were noted for eight males and twelve females receiving 300 ppm, for one male and three females receiving 30 ppm and for three males receiving 1.5 ppm. At 300 ppm in males, 5 of the 8 single cases of convulsions observed were encountered during the first week of treatment (one additional male rat during treatment week 3), while only one high-dose group female (out of 12 with convulsions) was observed with a convulsion during treatment week 1; one further female given 30 ppm exhibited a convulsion during week 1. The next reported case of convulsions occurred after 21 weeks of treatment or later. Four males and three females fed 300 ppm, one female given 30 ppm and one male at 1.5 ppm died following convulsive episodes. Irritability, overactivity, vocalisation, salivation and aggressive behaviour were recorded throughout the treatment period particularly in females given 300 ppm and, to a lesser degree, at 1.5 and 30 ppm. The number of thin males at 300 ppm was also higher than controls. Conversely, the number of obese ones was lower. There were no other treatment-related clinical signs during the dosing period. The neurological changes seen during the treatment period were not observed during the 'off-dose' Reversibility phase. Palpable masses There was no treatment-related effect upon the location, multiplicity or mean time of onset of palpable swellings.	**x** **x**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	Bodyweight (see Table A6.5-2) At 300 ppm, bodyweight gain of males and females was markedly lower than in controls, particularly during the first week of treatment (42% and 46% of controls in males and females, respectively). Weight gain continued to be significantly lower thereafter so that after 52 weeks of treatment, values were 85 and 82% of controls in males and females, respectively. At 30 ppm, overall male weight gain was slightly lower than controls throughout, but statistical significance was not achieved. In females, overall gains were similar to controls for the first 52 weeks but declined sharply thereafter. Consequently, values were statistically significant at the end of treatment. The decline may have been associated with high mortality seen in this group in comparison with others. There was no treatment-related effect on bodyweight gain at 0.5 and 1.5 ppm. During the reversibility phase, no clear improvement in weight gain was seen at 30 and 300 ppm following the cessation of treatment.	x
	Food consumption (see Table A6.5-3) At 300 ppm, food consumption was reduced during the first week of treatment compared with controls. Males were also affected during Week 2. There was no treatment-related effect on food intake at lower dose levels and overall consumption was broadly similar in all groups. As expected, food conversion at 300 ppm was markedly lower than in controls during Week 1 but was similar thereafter. Food conversion at other dose levels was unaffected.	x
	Achieved intake of test material (see Table A6.5-4) The estimated overall intakes of Fipronil are shown in Table A6.5-3. Predictably, the achieved intake in all groups generally declined with time, reflecting the changing ratio between food intake and bodyweight.	x
	Hematology (see Table A6.5-5) At 300 ppm, in general, packed cell volume, hemoglobin concentration and erythrocyte count were lower than in controls. Occasionally, these were associated with low mean cell hemoglobin and mean cell volume. Similar responses were often seen at 30 ppm and occasionally at 1.5 ppm. However, no such findings were detected after 12 weeks of the reversibility phase. Prothrombin times were generally slightly shorter at 300 ppm, and intermittently in females given 30 ppm. Although values at these dose levels were still low in females after 12 weeks of the 'off-dose' Reversibility phase, there were no marked inter-group differences in treatment Weeks 88 or 90. A trend of higher platelet counts was detected at 300 ppm and in males given 30 ppm towards the end of the treatment period (after Weeks 76, 88 or 90). No treatment-related effects were found in the blood smears taken after Weeks 50, 76 and 88/90.	x
	Clinical chemistry (see Table A6.5-6) At 300 ppm, higher plasma cholesterol particularly in females, calcium and total protein concentrations, low albumin, high α_1- and α_2- and β-globulin levels and low albumin:globulin ratios were seen. After 12 weeks of the reversibility phase, these findings (apart from the high calcium) were seen in females alone. At 30 ppm, plasma cholesterol levels were occasionally high whilst high calcium concentrations were seen only after 24 weeks of treatment. Evidence of high plasma proteins (total protein, low albumin:globulin ratios,	x

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	high α-globulins and low albumin) was also found. At 1.5 ppm, similar alterations in plasma proteins were detected after 76 and 88 weeks of treatment. No other findings were considered to be of toxicological significance. Thyroid hormones (see Table A6.5-7) At 300 ppm, low serum triiodothyronine (T_3) concentration was found in males but only after the first week of treatment. Following the cessation of treatment, T_3 was high after 4, 7 and 11 weeks of the 'off-dose' Reversibility phase in the 300 ppm females and after 7 and 11 weeks in the 30 ppm females. Circulating thyroxine (T_4) levels were consistently and dose-relatedly low in all groups of treated males, except at 0.5 ppm. Low T_4 levels were also seen in females given 300 ppm and occasionally in all other treated groups. But, at 300 ppm, no T_4 was detectable in serum after the first week of treatment. During the reversibility phase, there was no effect on T_4 in females. Recovery was evident in males given 1.5 and 30 ppm and was complete in all male groups after 11 weeks. Thyroid stimulating hormone (TSH) levels were markedly high at each sampling time during treatment with 300 ppm and on three occasions in males at 30 ppm. Once treatment stopped, recovery was immediate except in the high dose males where, although some recovery was evident, it was still incomplete at the end of the reversibility period. Urinalysis (see Table A6.5-8) At 300 ppm, the urinary pH was slightly low in both sexes throughout the treatment period, apart from in males during the Week 1 of dosing. At 30 ppm, low pH was also seen in males in the second half of the study. Urinary protein levels were high in both sexes at 300 ppm, and in males at 30 ppm. High urinary volumes, associated with low specific gravity, were found after 87 weeks in males at both 30 and 300 ppm. No reversibility of either low urinary pH or of protein levels was evident after 6 and 11 weeks of the 'off-dose' reversibility phase. Organ weights (see Table A6.5-9) At 300 ppm, absolute and bodyweight-relative liver and thyroid weights were significantly increased in both sexes after 52 and 89/91 weeks of treatment. In the liver, absolute and bodyweight-relative weights were also increased at 30 ppm. Absolute and bodyweight-relative kidney and adrenal weights at 30 and 300 ppm. In males fed 300 ppm, absolute and bodyweight-relative spleen weights were increased after 89/91 weeks of treatment. After 13 weeks of the 'off-dose' Reversibility phase, bodyweight-relative kidney and liver weights were still increased at 300 ppm. All other findings were considered not to be treatment-related. Macroscopic pathology (see Table A6.5-10) At the 52-week interim kill (chronic toxicity phase), large livers were found in four males and one female at 300 ppm and enlarged thyroids in one male at both 30 ppm and 300 ppm. At the 89/91 week terminal kill (oncogenicity phase), higher incidences of large and pale kidneys occurred in both sexes at 30 and 300 ppm. Incidences of granular kidneys, large livers and large thyroids were also high in males given 300 ppm, and to a lesser extent in the high dose females and in males fed 30 ppm. Decedent males at 300 ppm also had higher incidences of large and/or pale kidneys and large livers, adrenals and thyroids. In animals killed at the end of the 13-week 'off-dose' reversibility phase,	**x** **x** **x** **x**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	there were no clearly treatment-related findings although large kidneys were seen occasionally in males at 30 and 300 ppm and two males at 300 ppm had large livers. Histopathology, non-neoplastic findings (see Table A6.5-11) The only finding of toxicological significance was progressive senile nephropathy seen in all phases of the study. Progressive senile nephropathy occurred in all groups, although the incidence and severity was greatest in chronic toxicity phase rats given 300 ppm and in the oncogenicity phase animals fed 30 and 300 ppm; it was most marked in males. The finding was still apparent at the end of the 13-week 'off-dose' Reversibility phase where its incidence was higher in females given 30 and 300 ppm whilst severity was greatest in rats fed 1.5 ppm and above. <u>Histopathology, neoplastic findings</u> (see Table A6.5-12) Thyroid follicular cell tumours were the only treatment-related neoplastic lesions. Considering all animals from the oncogenicity phase together, irrespective of the time of death, increased incidences were found of benign follicular cell adenomas in both sexes and of malignant follicular cell carcinomas in males alone at 300 ppm. Incidences of follicular cell tumours at lower dose levels were well within the historical control range of concurrently run studies at the same laboratory (see Table A6.5-13). No treatment-related neoplastic lesions were found in rats from the chronic toxicity phase. Six animals from the 13-week 'off-dose' reversibility phase had thyroid follicular cell tumours. **Discussion** In this combined chronic toxicity and oncogenicity study in rats with dose levels of 0, 0.5, 1.5, 30 and 300 ppm of Fipronil, mortality occurred in the early weeks at the highest dose level following convulsive episodes. This, together with the marked depression in bodyweight of these animals, showed that 300 ppm exceeded the maximum tolerated dose. The depression in bodyweight, which was particularly marked during Week 1 and associated with reduced food conversion, was considered to be a non-specific indicator of toxicity. Central nervous system involvement was shown by the behavioural changes (irritability, overactivity, vocalisation, aggression and convulsive episodes). However, in the absence of any associated histopathological findings and any behavioural changes following cessation of treatment, these were considered pharmacological responses rather than pathological changes. Hepatomegaly, associated with changes in plasma protein and cholesterol, and shorter prothrombin times, was indicative of altered liver function that persisted into the 'off-dose' Reversibility phase. Such changes are a common adaptive metabolic response to a xenobiotic and are not of direct toxicological importance. The increased thyroid weights at 30 and 300 ppm were associated with elevated circulating levels of thyroid stimulating hormone (TSH). Reduced thyroxine (T_4) levels were seen in all treated groups, although the level of the more physiologically active triiodothyronine (T_3) was only reduced during the first week of treatment in males at 300 ppm. These changes were secondary to the treatment-related	**x** **x**

Section A6.5 **Annex Point IIA, VI.6.5**	**Chronic toxicity and carcinogenicity study in rats**	
	enhanced liver metabolic activity, and hence, increased metabolism of thyroid hormones. The reduction in plasma hormone levels disturbed the negative feedback to the pituitary stimulating increased release of TSH and, in turn, enhanced thyroid activity. Thus, the high incidence of follicular cell tumours at 300 ppm in the oncogenicity phase resulted from continuous stimulation of the thyroid. During the 'off-dose' reversibility phase when treatment had ceased, TSH and thyroid hormone levels returned to normal in all groups, apart from TSH in males given 300 ppm where recovery was in progress. The thyroid gland in rats is particularly sensitive to disturbances in thyroid hormones compared to the human thyroid. Therefore the effects in this study, particularly the increased incidence of follicular cell tumours at the highest concentration, may have no relevance to the risk to humans from exposure to Fipronil. The renal findings (high kidney weights, macroscopically enlarged and pale kidneys) were a likely consequence of the higher incidence and severity of progressive senile nephropathy at 30 and 300 ppm and indicative of a possible enhancement of this spontaneous age-related change by Fipronil. The higher urinary protein excretion was also probably related to this. However, the rationale for the slightly higher plasma calcium levels and low urinary pH at these same dose levels is unclear but could be related too. Since the high spleen and adrenal weights had no histopathological correlates, they were considered to be of no toxicological significance.	
5.3 Conclusion	Dietary administration of 30 or 300 ppm of Fipronil to rats for up to 91 weeks produced functional and morphological changes in the liver, thyroid and kidneys and functional effects alone in the nervous system. In addition, an increased incidence of throid gland tumours was observed at 300 ppm, which was considered to have resulted from continued growth stimulation via elevated thyroid-stimulating hormone (TSH) levels, i.e. from a non-genotoxic mechanism. A dose-related incidence of convulsive episodes was noted in males at and above 1.5 ppm and in females at and above 30 ppm; in some cases, the convulsions were fatal. Slight decreases of circulating T_4 were observed at 0.5 ppm and 1.5 ppm. In the absence of any morphological alterations and of any concurrent effects on T3 and TSH serum concentrations, these effects are not considered to be of toxicological significance. The No Observed Adverse Effect level (NOAEL) was 0.5 ppm, corresponding to 0.019 mg/kg bw/d in males and 0.025 mg/kg bw/d in females, respectively.	
5.3.1 LO(A)EL	1.5 ppm, corresponding to 0.019 and 0.025 mg/kg bw/d in males and females, respectively.	**X**
5.3.2 NOAEL	0.5 ppm, corresponding to 0.059 and 0.078 mg/kg bw/d in males and females, respectively.	**X**
5.3.3 Other	none	
5.3.4 Reliability	1	
5.3.5 Deficiencies	No	

~~Table A6.5-1~~ Table A6.5.1-1 Group incidence of clinical signs – Oncogenicity phase (number of animals affected)

X

Finding	Dose level (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Thin build	14	8	12	14	22	9	11	13	18	9
Obese	9	8	6	4	0	4	5	4	2	0
Convulsion	0	0	3	1	5	0	0	0	2	11
Aggressive	0	3	1	2	4	0	0	4	2	9
Irritable	3	4	3	4	6	2	2	5	8	18
Overactive	0	0	0	0	~~0~~ 1	0	0	1	0	3
Vocalisation	4	9	3	6	10	4	2	11	7	19
Salivation	0	0	1	0	1	0	0	0	2	8

X

Group size 50♂ and ♀

~~Table A6.5-2~~ Table A6.5.1-2 Group mean bodyweight change (g)

X

Weeks	Dose level (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Treatment period										
0-1	62	62	61	58**	26**	28	29	27	25*	13**
1-52	650	666	646	612	578**	310	321	312	306	265**
0-52	712	728	707	670	603**	338	350	339	330	278**
0-88/90	699	771)	781)	652	576**	451	420	438	346*	339**
(% of control)	(100)	~~(110~~ 100	(112	(93)	(82)	(100)	(93)	(97)	(77)	(75)
Reversibility period										
52-64	-12	-10	37	-4	3	36	50	30	42	21

X

Male values: Weeks 0-88; Female values: Weeks 0-90; Percent of control in parenthesis
Statistical evaluation: * $p \leq 0.05$ ** $p \leq 0.01$

~~Table A6.5-3~~ Table A6.5.1-3 Group mean food consumption (g/animal/week)

X

Weeks	Dose level (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
1 (% control)	196 (100)	198 (108)	195 (99)	193 (98)	146 (74)	152 (100)	152 (100)	154 (101)	149 (98)	119 (78)
2 (% control)	206 (100)	205 (99)	200 (96)	203 (98)	186 (89)	154 (100)	155 (101)	155 (101)	156 (101)	149 (97)
1 – 89/90 (% control)	18098 (100)	18000 (99)	17749 (98)	18677 (103)	17614 (97)	13931 (100)	14027 (101)	14043 (101)	14020 (101)	13700 (98)

Male values Weeks 1 – 89: Female values Weeks 1 – 90

~~**Table A6.5-4**~~ **Table A6.5.1-4** **Group mean test material intake (mg/kg bw/d)** X

Weeks	Dose level (ppm)							
	Males				Females			
	0.5	1.5	30	300	0.5	1.5	30	300
1-90	0.019	0.059	1.27	12.68	0.025	0.078	1.61	16.75

~~**Table A6.5-5**~~ **Table A6.5.1-5** **Group mean hematology** X

Parameter	Week	Dose level (ppm)									
		Males					Females				
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
PCV (%)	24	46	45	45	45	43**	44	45	45	44	41**
	50	47	47	47	46	43***	46	45	44*	43***	41***
	76	47	44	42*	41*	42*	43	42	44	42	39*
	88/90	46	46	46	42	41*	42	44	44	42	39
	12R	48	49	46	46	48	45	47*	46	45	44
Hb (g%)	24	16.1	16.0	16.1	15.7	15.0***	15.5	15.9	15.8	15.3	14.7**
	50	16.1	16.1	16.1	15.7	14.9***	15.7	15.7	15.2	15.1*	14.2***
	76	16.2	15.1	14.6	14.0*	14.4*	15.0	14.8	15.5	14.4	13.6*
	88/90	15.6	15.3	15.1	13.9*	13.7*	14.2	15.1	15.1	14.5	13.1
	12R	15.7	15.9	15.3	15.0	15.7	14.8	15.5*	15.0	15.0	14.5
RBC (10^6/mm³)	24	9.37	9.23	9.01*	8.95*	8.90*	8.11	8.41	8.37	8.14	8.11
	50	9.26	9.10	8.97	8.82	8.76*	8.15	8.16	8.01	7.90	7.87
	76	9.01	8.60	8.14	7.96*	8.68	8.02	7.82	8.32	7.82	~~8.58~~ 7.58 X
	88/90	8.80	8.61	8.75	8.00	8.14	7.73	8.05	8.17	7.95	7.47
	12R	9.05	9.25	8.69	8.75	8.71	7.93	8.20	7.82	8.05	7.86
MCH (pg)	24	17	17	18	18	17	19	19	19	19	18***
	50	18	18	18	18	17	20	19	19	19	18***
	76	18	18	18	18	17***	19	19	19	18	18
	88/90	18	18	17	17	17	18	19	19	18	18
	12R	17	17	18	17	18	19	19	19*	19	19
MCV (cμ)	24	49	49	51	50	48	54	53	54	54	51***
	50	51	52	53	52	50	57	56	55*	55*	52***
	76	52	51	52	51	49**	54	54	53	53	53
	88/90	53	54	53	52	50	54	55	54	53	54
	12R	53	53	54	53	55	57	58	58	56	56
PT (secs)	24	14.1	14.6*	14.0	14.1	13.6*	13.4	13.8*	13.5	13.0*	12.7**
	50	15.2	16.0	15.0	15.5	14.7	14.3	14.9	14.1	13.9	12.9**
	76	13.5	14.1	13.7	13.3	13.0	13.4	12.3***	12.7*	12.2***	12.0***
	88/90	13.1	14.1*	14.0*	13.1	13.0	12.1	12.2	11.9	11.9	11.8
	12R	14.0	14.9*	14.1	14.3	14.1	13.2	13.7	13.2	12.7*	12.5*
Platelets (1000/cmm)	76	1042	1048	1121	1296*	1246*	852	1058*	958	1021	1193***
	88/90	918	917	1050	1185*	1338***	921	1133*	956	1017	1061

Male values: Week 88; Female values: Week 90
12R: values after 12 weeks of the 'off-dose' Reversibility phase following 52 weeks of treatment
Statistical evaluation: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

~~**Table A6.5-6**~~ **Table A6.5.1-6** **Group mean clinical chemistry** X

Parameter	Week	Dose level (ppm)									
		Males					Females				
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
Cholesterol (mg%)	24	56	67	65	69	82*	76	66	63	79	135***
	50	88	82	103	102	117	113	101	114	137	229***
	76	104	125	140	135	149*	95	169*	128	169*	228***
	88/90	134	127	135	174	170	143	170	178	231*	230*
	12R	117	110	122	126	133	106	100	107	128	210***
Calcium (mmol/l)	24	2.5	2.5	2.5	2.6**	2.6**	2.5	2.6	2.5	2.6**	2.7***
	50	2.7	2.6	2.7	2.7	2.8*	2.7	2.7	2.7	2.7	2.8***
	76	2.6	2.7	2.7	2.8*	2.7	2.7	2.7	2.7	2.7	2.9**
	88/90	2.7	2.6	2.6	2.7	2.8	2.8	2.8	2.8	2.7	2.9*
	12R	2.6	2.6	2.6	2.7	2.6	2.6	2.6	2.6	2.7	2.7**
Total protein (mmol/l)	24	6.8	6.9	6.8	7.1*	7.4***	7.3	6.9	7.3	7.6	~~7~~ 7.8* X
	50	6.9	6.8	6.8	6.9	7.3***	7.4	7.4	7.7	8.0***	8.2***
	76	6.7	6.7	6.7	6.9	6.9	7.2	7.5	7.3	7.5	8.1***
	88/90	7.5	7.1	7.2	7.3	7.5	~~8.5~~ 8.0	8.2	8.1	8.2	8.5* X
	12R	7.2	7.0	7.0	7.1	7.1	7.8	7.6	7.8	7.9	8.2*
Albumin	24	2.9	2.9	2.9	2.9	2.5***	3.9	3.6	3.7	3.8	3.6
	50	2.8	3.0	2.7	2.8	2.7	3.7	3.9	3.8	3.8	3.5
	76	2.9	2.5*	2.5*	2.5**	2.4**	3.5	3.2	3.3	3.1	3.0*
	88/90	3.1	2.9	2.7**	2.4***	2.3***	3.8	3.5	3.4	3.0**	3.0**
	12R	2.9	2.8	2.7	2.5**	2.6	3.4	3.7	3.7	3.8	3.2
A:G ratio	24	0.8	0.7	0.7	0.7*	0.5***	1.1	1.1	1.0	1.0*	0.9***
	50	0.7	0.8	0.6	0.7	0.6**	1.0	1.1	1.0	0.9	0.8***
	76	0.7	0.6*	0.6*	0.6**	0.5***	0.9	0.8	0.9	0.8	0.6***
	88	0.7	0.7	0.6*	0.5***	0.5***	0.9	0.8	0.7*	0.6**	0.6***
	12R	0.7	0.7	0.6	0.6*	0.6	0.8	1.0	0.9	0.9	0.7
α_1 globulin	24	1.4	1.6	1.5	1.8**	1.9***	1.2	1.2	1.3	1.4	1.7***
	50	1.6	1.5	1.7	1.8	2.0**	1.4	1.3	1.5	1.9**	2.1***
	76	1.4	1.7*	1.7*	1.9**	1.9**	1.3	1.7	1.5	1.7	2.0**
	88	1.7	1.6	1.8	2.1*	2.3**	1.4	1.8	1.9*	2.3**	2.1**
	12R	1.6	1.6	1.8	1.9*	1.8	1.4	1.2	1.4	1.6	2.3***
α_2 globulin	24	0.5	0.5	0.5	0.5	0.6***	0.4	0.4	0.5	0.5*	0.5***
	50	0.4	0.4	0.5	0.5	0.5	0.5	0.5	0.5	0.6	0.8***
	76	0.4	0.4	0.4	0.5**	0.6***	0.5	0.6	0.6	0.6**	0.7***
	88	0.5	0.5	0.5	0.6	0.6*	0.7	0.7	0.7	0.8*	0.9***
	12R	0.5	0.4	0.4	0.5	0.6	0.6	0.5	0.5*	0.6	0.8*
β globulin	24	1.8	1.7	1.7	1.7	2.2***	1.5	1.4	1.5	1.6	1.7*
	50	1.8	1.7	1.8	1.7	2.0*	1.5	1.5	1.6	1.6	1.6
	76	1.8	1.9	1.9	1.9	1.8	1.6	1.8	1.7	1.8	2.0*
	88	2.0	2.0	2.1	2.1	2.1	1.9	1.9	1.8	1.9	2.2***
	12R	2.0	2.0	1.9	2.0	1.9	2.1	1.8	1.9	1.7*	1.7*

Male values: Week 88; Female values: Week 90
12R: values after 12 weeks of the 'off-dose' Reversibility phase following 52 weeks of treatment
Statistical evaluation: * $p<0.05$ ** $p<0.01$ *** $p<0.001$

~~**Table A6.5-7**~~ **Table A6.5.1-7** **Group mean thyroid hormone concentrations**

X

Parameter	Week	Dose level (ppm)									
		Males					Females				
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
T_3 (ng/ml)	1	0.61	0.59	0.61	0.58	0.53*	0.78	0.75	0.77	0.75	0.80
	4	0.75	0.83	0.85	0.84	0.72	0.77	0.83	0.75	0.70	0.81
	12	0.92	0.91	0.95	1.10*	1.01	1.17	1.17	1.11	1.03	1.17
	24	0.69	0.73	0.74	0.70	0.64	0.93	0.92	0.84	0.82	0.84
	50	0.70	0.67	0.84*	0.82*	0.69	0.87	0.88	0.83	0.84	0.87
	2R	0.64	0.58	0.64	0.69	0.62	0.98	0.87	0.95	0.89	1.10
	4R	0.70	0.59	0.68	0.74	0.77	0.88	0.91	0.88	0.95	1.13***
	7R	0.80	0.77	0.78	0.81	0.89	0.91	1.03	0.87	1.10*	1.19**
	11R	0.56	0.52	0.59	0.62	0.64	0.75	0.81	0.83	0.95**	1.11***
T_4 (µg/dl)	1	2.93	3.02	2.23*	1.16***	0.00***	2.32	1.86	2.58	1.26**	0.00***
	4	3.14	2.70*	2.56**	1.84***	0.39***	3.03	2.48*	2.36*	1.46***	0.79***
	12	5.18	4.74	3.96**	3.50***	1.22***	3.62	2.85**	2.87*	2.05***	1.10***
	24	4.58	3.81*	3.35***	2.43***	0.76***	2.85	3.09	3.49**	2.98	1.46***
	50	5.95	5.51	4.83**	3.90***	2.07***	3.31	3.46	3.00	2.06***	1.38***
	2R	3.97	3.71	3.45	3.05**	2.56***	1.67	1.70	1.27	1.39	1.59
	4R	4.10	3.45*	2.79***	2.64***	3.21**	2.54	2.53	2.05	2.04	2.21
	7R	3.80	3.31	2.94*	2.66**	3.12	2.16	2.30	1.71*	2.04	1.85
	11R	3.70	3.58	3.25	3.29	3.52	2.95	3.60*	3.27	3.65*	3.09
TSH (ng/ml)	1	4.7	7.1	6.2	11.8***	20.3***	3.5	3.5	3.2	3.6	7.6***
	4	5.2	8.0	6.5	11.2**	22.9***	3.8	3.9	3.3	3.9	7.5***
	12	5.7	7.2	5.8	6.1	18.4***	3.4	3.4	2.9	3.5	8.7***
	24	7.2	10.0	6.9	8.6	21.0***	3.2	3.7	3.2	3.9	6.6***
	50	13.0	17.1	12.4	26.6*	57.3***	6.2	8.0	5.5	6.1	13.5***
	2R	7.2	7.1	5.7	7.4	12.7**	3.5	3.6	3.5	3.5	3.8
	4R	5.6	6.4	6.0	7.3	10.8**	3.7	3.5	3.6	3.3	3.7
	7R	5.9	6.3	4.4	5.2	9.1*	3.0	3.2	3.1	2.7	3.1
	11R	3.8	4.6	4.6	5.1	8.4**	2.7	3.1	2.9	2.7	3.1

2R-11R: values for the specified weeks of 13-week 'off-dose' Reversibility phase following 52 weeks of treatment
* $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

~~Table A6.5-8~~ Table A6.5.1-8 **Group mean urinalysis**

X

Parameter	Week	Dose levels (ppm)									
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
		Males					Females				
Volume (ml)	23	9.5	6.0	6.5	6.5	6.0	4.0	4.5	5.0	5.0	4.0
	49	11.0	9.5	10.5	10.5	12.5	7.5	7.5	8.5	7.5	8.0
	76	9.0	8.5	11.5	12.0	12.5	8.0	11.0	9.5	12.5*	11.0
	87/90	9.5	10.0	12.5	17.5**	16.5*	11.0	11.0	13.0	11.0	11.0
	6R	12.5	10.0	9.5	14.5	9.0	8.5	9.0	11.5	8.5	8.5
	11R	12.0	8.5	10.0	16.0	11.5	9.0	8.5	8.0	7.5	7.0
pH	23	6.6	6.6	6.7	6.4	6.5	6.4	6.4	6.3	6.4	6.2*
	49	6.6	6.6	6.5	6.4	6.4	6.3	6.1	6.0*	6.2	5.9**
	76	6.3	5.9**	6.1	5.9~~b~~ **	6.0*	6.1	5.9	5.9	6.0	5.8
	87/90	6.0	6.0	6.1	5.7*	5.7*	6.1	6.0	6.3	5.8	5.8
	6R	6.6	6.5	6.4	6.2**	6.1***	6.2	6.2	6.1	6.1	5.9**
	11R	6.6	6.4*	6.4	6.1***	6.2***	6.2	6.3	6.4	6.0	5.9**
Specific gravity	23	1050	1060	1060	1067*	1061	1045	1052	1047	1046	1056
	49	1045	1044	1039	1045	1044	1037	1039	1036	1035	1043
	76	1047	1052	1042	1038	1041	1035	1037	1035	1029	1036
	87/90	1048	1046	1040	1033***	1034***	1031	1034	1028	1035	1037
Protein (median mg%)	23	100	100	100	100	100	40	10	10	10	100
	49	300	200	150	525	1000	400	125	200	400	1000
	76	1000	1000	1000	1000	1000	100	1000	550	600	1000
	87/90	1000	1000	1000	1000	1000	700	1000	700	1000	1000
	6R	950	450	1000	1000	1000	295	750	500	1000	1000
	11R	1000	1000	1000	1000	1000	550	1000	1000	1000	1000

X

6 – 11 R: values for the specified weeks of 13-week of the off dose reversibility phase following 52 weeks of treatment
Statistical analysis: * $p \leq 0.05$ ** $p \leq 0.01$ ** * $p \leq 0.001$

X

~~Table A6.5-9~~ Table A6.5.1-9 **Group mean organ weights**

Organ weight	Week	Dose level (ppm)									
		Males					Females				
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
Liver											
Abs. wt (g)	52	29.5	30.0	29.1	34.3	40.5**	16.4	17.3	17.1	19.1	23.4**
	89/91	28.3	32.4	32.1	33.9	39.4**	23.0	22.0	21.5	25.0	27.9*
	13R	30.3	30.3	32.9	30.9	30.6	18.5	19.4	17.8	21.0	19.7
Rel. wt (% bw)	52	3.53	3.44	3.40	4.32**	5.26**	3.56	3.62	3.70	4.31**	5.49**
	89/91	3.30	3.24	3.48	4.40**	5.41**	3.88	3.98	3.82	5.12**	6.14**
	13R	3.50	3.70	3.44	3.69	4.55*	3.42	3.60	3.54	3.81*	4.68**
Thyroid											
Abs. wt (g)	52	0.039	0.035	0.042	0.047	0.056**	0.027	0.031	0.030	0.032	0.045**
	89/91	0.042	0.051*	0.053*	0.063**	0.094**	0.036	0.038	0.036	0.044	0.072*
	13R	0.038	0.039	0.043	0.045	0.045	0.031	0.031	0.030	0.034	0.035
Rel. wt (% bw)	52	0.0047	0.0040	0.0050	0.0058*	0.0073**	0.0059	0.0064	0.0065	0.0073*	0.0107**
	89/91	0.0049	0.0052	0.0056	0.0082**	0.0129**	0.0060	0.0070	0.0065	0.0090**	0.0156*
	13R	0.0045	0.0047	0.0045	0.0054	0.0067*	0.0059	0.0058	0.0061	0.0063	0.0080**
Kidney											
Abs. wt (g)	52	5.81	5.40	6.26	6.18	5.87	3.78	3.59	3.50	3.76	3.89
	89/91	6.32	7.29	7.24	8.56**	9.86*	4.23	4.51	4.15	5.75	4.89
	13R	6.03	6.01	6.80	8.22	6.20	3.92	3.72	3.71	4.16	4.32
Rel. wt (% bw)	52	0.702	0.627	0.732	0.799	0.764	0.831	0.764	0.763	0.850	0.916
	89/91	0.737	0.741	0.791	1.144**	1.354**	0.716	0.829*	0.751	1.207**	1.119**
	13R	0.719	0.745	0.716	0.994	0.940	0.742	0.701	0.744	0.774	1.038*
Adrenal											
Abs. wt (g)	52	0.063	0.060	0.068	0.068	0.067	0.090	0.089	0.087	0.078	0.096
	89/91	0.074	0.088	0.086	0.092	0.109**	0.108	0.138	0.123	0.125	0.128
	13R	0.065	0.063	0.063	0.068	0.065	0.092	0.191	0.097	0.092	0.097
Rel. wt (% bw)	52	0.0076	0.0069	0.0081	0.0092	0.0088	0.0198	0.0192	0.0190	0.0178	0.0229
	89/91	0.0086	0.0088	0.0094	0.0124*	0.0155*	0.0188	0.0256	0.0222	0.0262*	0.0299*
	13R	0.0077	0.0078	0.0066	0.0082	0.0098	0.0177	0.0316	0.0199	0.0173	0.0233*
Spleen											
Abs. wt (g)	52	1.229	1.161	1.276	1.121	1.248	0.710	0.714	0.669	0.646	0.647
	89/91	1.224	1.939*	1.540*	1.790	1.558*	0.858	0.987	0.822	0.918	0.876
	13R	1.235	1.244	1.374	1.515	1.176	0.908	0.812	0.831	0.769	0.714
Rel. wt (% bw)	52	0.1471	0.1333	0.1498	0.1413	0.1630	0.1561	0.1502	0.1455	0.1471	0.1529
	89/91	0.1416	0.1963	0.1633	0.2378	0.2122**	0.1453	0.1802*	0.1468	0.1900**	0.1939**
	13R	0.1407	0.1505	0.1440	0.1812*	0.1757	0.1677	0.1523	0.1673	0.1411*	0.1692

13R: values at end of 13-week 'off-dose' Reversibility phase following 52 weeks of treatment
Statistical evaluation: * $p \leq 0.05$ ** $p \leq 0.01$

~~Table A6.5-10~~ Table A6.5.1-10 **Group incidence of macroscopic findings**

X

Week of Kill	Finding	Dose Level (ppm)									
		Males					Females				
		0	0.5	1.5	30	300	0	0.5	1.5	30	300
52	Number examined	12	14	14	15	12	14	14	14	14	13
	Large liver	0	0	0	0	4	0	0	0	0	1
	Large thyroids	0	0	0	1	1	0	0	0	0	0
89/91	Number examined	20	14	22	20	12	23	21	21	13	22
	Large kidneys	3	2	6	11*	10**	0	2	0	5**	2
	Pale kidneys	1	1	3	8*	7**	0	2	0	4*	4*
	Granular kidneys	2	1	4	8	8**	0	1	0	4	4
	Large liver	1	0	0	3	6**	1	1	0	0	3
	Large thyroids	0	0	1	3	5**	1	0	0	1	3
13R	Number examined	13	15	13	13	10	13	13	11	15	10
	Large liver	0	1	0	0	2	0	0	0	0	0
	Large kidneys	0	0	1	3	2	0	0	0	0	0

X

13 R: values at end of 13-week of the off dose reversibility phase following 52 weeks of treatment
* p≤0.05 ** p≤0.01 ** * p≤0.00

~~Table A6.5-11~~ Table A6.5.1-11 **Group incidence of non-neoplastic microscopic findings – progressive senile nephropathy**

X

Phase of study/ Week of Kill	Dose Level (ppm)									
	Males					Females				
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
Phase of study										
Oncogenicity	26/50	2/50	32/50	42/50*	44/50** *	14/50	21/50	17/50	31/50**	24/50
Toxicity	6/15	3/15	5/15	7/15	11/15	4/15	6/15	4/15	6/15	8/15
Reversibility	8/15	7/15	9/15	8/15	9/15	5/15	4/15	7/15	11/15	13/15**
Week of kill										
Week 52	5/12	3/14	5/14	7/15	9/12	4/14	5/14	3/14	6/14	7/14
Week 89/91	8/20	11/14*	17/22*	17/20**	11/12**	6/23	12/21	5/21	9/13*	12/22
Week 13R	7/13	7/15	7/13	8/13	6/10	5/13	4/13	5/11	11/15	9/10

13 R: values at end of 13-week of the off dose reversibility phase following 52 weeks of treatment
* p≤0.05 ** p≤0.01 ** * p≤0.001

~~**Table A6.5-12**~~ **Table A6.5.1-12** **Group incidence of thyroid follicular cell tumours**

X

Type of neoplasm	Dose level (ppm)									
	0	0.5	1.5	30	300	0	0.5	1.5	30	300
	Males					Females				
Oncogenicity phase										
Malignant follicular cell carcinoma	0/49 (0)	0/48 (0)	0/50 (0)	0/50 (0)	5/50* (10)	0/50 (0)	1/50 (2.0)	0/50 (0)	1/50 (2.0)	2/50 (10)
Benign follicular cell adenoma	0/49 (0)	1/48 (2.1)	5/50* (10)	3/50 (6)	12/50*** (24)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	8/50** (16)
Total tumours	0/49 (0)	1/48 (2.1)	5/50* (10)	3/50 (6)	17/50*** (34)	0/50 (0)	1/50 (2)	0/50 (0)	1/50 (2)	10/50*** (20)
Reversibility phase										
Malignant follicular cell carcinoma	0/15 (0)	0/15 (0)	0/15 (0)	1/14 (7.1)	1/12 (8.3)	0/14 (0)	0/13 (0)	0/15 (0)	0/15 (0)	0/14 (0)
Benign follicular cell adenoma	0/15 (0)	0/15 (0)	0/15 (0)	0/14 (0)	1/12 (8.3)	0/14 (0)	0/13 (0)	1/15 (6.7)	0/15 (0)	2/14 (14.3)
Total tumours	0/15 (0)	0/15 (0)	0/15 (0)	1/14 (7.1)	2/12 (16.7)	0/14 (0)	0/13 (0)	1/15 (6.7)	0/15 (0)	2/14 (14.3)

Percent incidence in parenthesis
Statistical evaluation: * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

~~**Table A6.5-13**~~ **Table A6.5.1-13** **Historical control incidence of thyroid tumours**

X

Type of tumour	Sex	
	Males	Females
Malignant follicular cell carcinoma	0 – 6%	0 -10%
Benign follicular cell adenoma	2 – 10%	0 – 4%

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.1 Description: or off-white 3.2.5 Age/weight at study initiation/5.1 Materials and methods: ~~*Weight 189 to 192 g (males) and 158 to 162 g (females)*~~ Weight 155 to 231 g (males) and 127 to 195 g (females) 3.2.7 Control animals: ~~*No*~~ Yes (15 males and 15 females for the toxicity study, 15 males and 15 females for the reversibility study and 50 males and 50 females for the oncogenicity study) 3.3.1 Duration of treatment: ~~*104*~~ 53 weeks for the toxicity phase, 52 weeks for the reversibility phase, 89 weeks (males) or 91 weeks (females) for the oncogenicity phase. 3.4.4 Water consumption: *yes* daily 3.4.5 Ophthalmoscopic examination *: Yes: prior to start of treatment (all animals)* ~~*after 50 weeks and after 13 weeks without substance administration following 50 wk treatment ("reversibility" controls and high dose group only)*~~ After 50 weeks of treatment all surviving animals from control and high dose groups of the toxicity and reversibility phases were similarly examined. Surviving animals from control and high dose groups of the oncogenicity phase were similarly examined after 87 and 90 weeks of treatment for males and females respectively. 3.4.6 Haematology/3.4.7 Clinical chemistry: *Time points:* after *24, 50* (toxicity phase animals) and after *76, 88(♂) and 90(♀) weeks* (oncogenicity phase animals) *and after 12 weeks of reversibility following 52-week treatment* 3.4.7 Clinical chemistry: thyroid hormones: after 1, 4, 12, 24 and 50 weeks of treatment and after 2, 4, 7 and 11 weeks of the reversibility period blood samples were obtained from 10 male and 10 female reversibility phase rats. Parameters: triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH). 3.4.8 Urinalysis: *Time points:* After *23, 49* (toxicity phase animals) and after *75, 87(♂) and 90(♀) weeks* (oncogenicity phase animals) *and after 6 and 11 weeks* of the reversibility period (*off-treatment following 52-week treatment ("reversibility"))* 3.5.2 Gross and histopathology: Histopathology: *All dose groups* from animals killed or dying after Week 57/Microscopy: *high dose group and controls, other dose groups only if* macroscopic examination was abnormal ~~*effect*~~*s*
Results and discussion	Agree with applicant's version. **Revisions/amendments:**

	4.1.1 Clinical signs/5.2 Results and discussion: ~~(See Table A6.5-1)~~ (See Table A6.5.1-1) 4.1.2 Mortality: *However, no similar effect was noted among females receiving ~~200 ppm~~* 300 ppm *and the observation* at 30 ppm *is therefore considered to have arisen by chance.* 4.2 Body weight gain/5.2 Results and discussion: ~~(See Table A6.5-2)~~ (See Table A6.5.1-2) 4.3 Food consumption and compound intake/5.2 Results and discussion: ~~(See Tables A6.5-3 and 4)~~ (See Tables A6.5.1-3 and 4) 4.5.1 Haematology/5.2 Results and discussion: ~~(See Table A6.5-5)~~ (See Table A6.5.1-5) 4.5.2 Clinical chemistry/5.2 Results and discussion: ~~(See Table A6.5-6)~~ (See Table A6.5.1-6) 4.5.3 Urinalysis/5.2 Results and discussion: ~~(See Table A6.5-8)~~ (See Table A6.5.1-8) 4.6.1 Organ weight/5.2 Results and discussion: ~~(See Table A6.5-9)~~ (See Table A6.5.1-9) 4.6.2 Gross and histopathology/5.2 Results and discussion: ~~(See Table A6.5-10)~~ (See Table A6.5.1-10) *Micropathology:* ~~(See Tables A6.5-11, 12 and -13)~~ (See Tables A6.5.1-11, -12 and -13) ~~*a malignant chardoma.*~~ a malignant chordoma. 4.7 Other/5.2 Results and discussion: ~~(See Table A6.5-7)~~ (See Table A6.5.1-7) T4 levels were also consistently low for females receiving 300 ppm and occasionally low for females receiving 0.5, 1.5 or 30 ppm. The effect was particularly marked after the first week for animals receiving 300 ppm, when no T4 was detectable in the serum. During the reversibility period T4 levels remained lower than control levels after 2 weeks for males which had received 30 or 300 ppm, after 4 weeks for all previously treated males and after 7 weeks of recovery for males which had received 1.5 or 30 ppm. Dosage-relationship was not apparent on the latter two occasions. No effects on T4 concentration were apparent among males after 11 weeks of recovery or among the females on any occasion during the reversibility period. The concentration of TSH was markedly higher than that of the controls on each occasion of examination during the treatment period for animals receiving 300 ppm and on three occasions in males receiving 30 ppm. After withdrawal of treatment TSH levels of females which had received 300 ppm and males which had received 30 ppm showed immediate recovery; in males which had received 300 ppm recovery was evident but was incomplete by the end of the reversibility period.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: ~~*on all surviving animals from the control and highest dose level from the chronic toxicity phase after 50 weeks of treatment*~~

	~~*and at the end of the 13-week reversibility phase.*~~ After 50 weeks of treatment all surviving animals from control and high dose groups of the toxicity and reversibility phases were similarly examined. 5.2 Results and discussion: *104~~+~~±12%, 102~~+~~±13.5%, 95.7~~+~~±4.2% and 98.4~~+~~±3.4% of nominal at 0.5, 1.5, 30 and 300 ppm, respectively.* *The achieved concentrations were within +15% of nominal.* This value is not found in the study report in the document IV. *Achieved intake*: *The estimated overall intakes of Fipronil are shown in ~~Table A6.5-3~~* Table A6.5-4. *Thyroid hormones: ~~Circulating thyroxine (T_4) levels were consistently and dose relatedly low in all groups of treated males, except at 0.5 ppm. Low T_4 levels were also seen in females given 300 ppm and occasionally in all other treated groups. But, at 300 ppm, no T_4 was detectable in serum after the first week of treatment. During the reversibility phase, there was no effect on T_4 in females. Recovery was evident in males given 1.5 and 30 ppm and was complete in all male groups after 11 weeks.~~* T4 levels were also consistently low for females receiving 300 ppm and occasionally low for females receiving 0.5, 1.5 or 30 ppm. The effect was particularly marked after the first week for animals receiving 300 ppm, when no T4 was detectable in the serum. During the reversibility period T4 levels remained lower than control levels after 2 weeks for males which had received 30 or 300 ppm, after 4 weeks for all previously treated males and after 7 weeks of recovery for males which had received 1.5 or 30 ppm. Dosage-relationship was not apparent on the latter two occasions. No effects on T4 concentration were apparent among males after 11 weeks of recovery or among the females on any occasion during the reversibility period. *Organ weight:* ~~Absolute and bodyweight relative kidney and adrenal weights at 30 and 300 ppm. In males fed 300 ppm, absolute and bodyweight relative spleen weights were increased after 89/91 weeks of treatment.~~ The absolute and bodyweight-relative kidney weights of animals receiving 30 or 300 ppm killed for terminal examination after 89 or 91 weeks were higher than control values, as were the adrenal weights of males receiving these concentrations. The absolute and bodyweight-relative spleen weights of males receiving 300 ppm wee also high compared with controls. Statistical significance was not attained in all cases. 5.3.1 LOAEL: *1.5 ppm, ~~corresponding to 0.019 and 0.025 mg/kg bw/d in males and females, respectively.~~* corresponding to 0.059 and 0.078 mg/kg bw/d in males and females, respectively. 5.3.2 NOAEL: *0.5 ppm, ~~corresponding to 0.059 and 0.078 mg/kg bw/d in males and females, respectively~~*. corresponding to 0.019 and 0.025 mg/kg bw/d in males and females, respectively
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	

Remarks

Section A6.6 **Genotoxicity studies**
Annex Point IIA, VI.6.6

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In v*itro gene mutation study in bacteria**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.6.1/01** XXXX Study to determine the ability of M&B 46030 to induce mutation in four histidine-requiring strains of *Salmonella typhimiurim*. (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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes: Not stated but compliant with OECD 471 (1981); EU 92/69/EEC (1992)	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	X
3.1.1 Lot/Batch number	IGB 438	
3.1.2 Specification	As given in section 2	X
3.1.2.1 Description	White crystalline solid	
3.1.2.2 Purity	95-97%	
3.1.2.3 Stability	Stable	X
3.2 Study Type	Bacterial reverse mutation test	
3.2.1 Organism/cell type	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100	
3.2.2 Deficiencies / Proficiencies	None specifically mentioned in the report	
3.2.3 Metabolic activation system	S9 mix (derived from Aroclor 1254-induced rat liver)	
3.2.4 Positive control	2-nitrofluorene (2NF), sodium azide (NaN_3), 9-aminoacridine (AAC), 2-aminoanthracene (AAN)	
3.3 Administration/ Exposure; Application of test substance		
3.3.1 Concentrations	0.8, 4, 20, 100, 500 µg/plate (mutation assay 1); 25, 50, 100, 200, 400 µg/plate (mutation assay 2)	
3.3.2 Way of application	Substance dissolved in Dimethyl sulfoxide (DMSO)	X
3.3.3 Pre-incubation time	-	

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In v*itro gene mutation study in bacteria**
3.3.4 Other modifications	None
3.4 Examinations	
3.4.1 Number of cells evaluated	Not applicable
	4. RESULTS AND DISCUSSION
4.1 Genotoxicity	
4.4.1 Without metabolic activation	No
4.4.2 With metabolic activation	No
4.2 Cytotoxicity	Yes: concentrations at and above 500 µg/plate
	5. APPLICANT'S SUMMARY AND CONCLUSION
5.1 Materials and methods	Histidine dependent auxotrophic mutant strains TA98, TA100, TA1535 and TA1537 of *Salmonella typhimurium* were exposed to Fipronil in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). Two independent mutation tests, using triplicate plates for the test substance and quintuplicate plates for negative (solvent) and positive controls, were conducted in both metabolic activation conditions. After two days of incubation at 37°C, the numbers of revertant colonies on the agar plates were scored using an image analysis system and the background lawn examined for signs of toxicity. The concentrations of Fipronil, diluted in Dimethyl sulfoxide (DMSO), were 0, 0.8, 4, 20, 100 and 500 µg/plate in mutation test 1 and 0, 25, 50, 100, 200 and 400 µg/plate in mutation test 2 in both the presence and absence of S-9 mix, respectively. The solvent control was DMSO. Dose levels for the mutation tests were selected on the basis of results of a preliminary toxicity test conducted with TA100 under both metabolic activation conditions with dose levels of 8, 40, 200, 1000 and 5000 µg/plate. The positive control compounds used in the absence of metabolic activation were 2-nitrofluorene (50 µg/plate with TA98), sodium azide (2 µg/plate with TA100 and TA1535) and 9-aminoacridine (50 µg/plate with TA1537). In the presence of metabolic activation 2-aminoanthracene (5 µg/plate with TA98 and TA100) was used.

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In v*itro gene mutation study in bacteria**

5.2	**Results and discussion**	(See Tables A6.6.1.1-1 and -2) In the preliminary toxicity test, toxicity was observed at 1000 and 5000 µg/plate in both the absence and presence of S9 mix. In the first mutation test, Fipronil was toxic at concentrations at 500 µg/plate. There were no significant increases in the numbers of revertant colonies in any strain in either the presence or absence of metabolic activation. The positive control compounds produced the expected increases in the numbers of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.
5.3	**Conclusion**	Fipronil showed no evidence of mutagenic activity in bacteria in either the presence or absence of metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6.6.1.1-1: Ames Test with Fipronil: Mean number of S. typhimurium revertants – Mutation assay 1

µg/plate	0	0.8	4	20	100	500	Historical control	
Without S9-mix							**Mean**	**Range**
TA 98	23.4	20.7	15.0	18.7	16.3	20.3 (S)	21	~~8~~ 6-46
TA 100	72.0	73.0	66.3	73.7	80.3	59.7 (S)	114	~~6~~ 66-178
TA 1535	19.8	21.0	22.0	20.7	26.7	17.0 (V)	14	2-37
TA 1537	10.8	9.0	13.0	23.5	11.3	13.7 (S)	8	1-19
With S9-mix								
TA 98	35.6	59.0	51.3	35.3	39.7	48.7 (S)	36	16-57
TA 100	131.4	138.0	117.0	122.7	136.7	140.7	143	83-219
TA 1535	19.2	11.3	12.7	17.7	9.3	8.7 (S)	20	6-46
TA 1537	10.0	14.0	~~11.0~~ 12.3	11.0	16.7	14.7 (S)	15	3-28

Historical control compiled over a 6-month period from at least 25 consecutive experiments.
(S) = Slight toxicity i.e. some thinning of background lawn and/or presence of microcolonies
(V) = Very thin background lawn

X

X

Table A6.6.1.1-2: Ames Test with Fipronil: Mean number of S. typhimurium revertants – Mutation assay 2

µg/plate	0	25	50	100	200	400	Historical control	
Without S9-mix							**Mean**	**Range**
TA 98	17.8	22.3	14.7	16.0	18.3	18.3	21	~~8~~ 6-46
TA 100	76.4	72.3	81.3	80.0	81.7	59.0	114	~~6~~ 66-178
TA 1535	16.2	13.7	14.0	16.3	12.7	19.7	14	2-37
TA 1537	8.2	10.3	9.3	9.7	12.7	8.7	8	1-19
With S9-mix								
TA 98	31.4	27.7	27.7	28.0	26.0	36.0	36	16-57
TA 100	107.4	107.7	118.0	103.3	115.3	123.0	143	83-219
TA 1535	23.0	25.3	22.3	23.0	22.3	19.3	20	6-46
TA 1537	11.4	14.0	15.7	11.3	14.7	12.7	15	3-28

Historical control compiled over a 6-month period from at least 25 consecutive experiments.

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *Salmonella ~~typhimiurim~~* typhimurium. *Microtest Research Limited;* UK 3.1 Test material: ~~*As given in section 2*~~ Fipronil MB 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.3.2 Way of application : *Dimethyl ~~sulfoxide~~* sulphoxide to give 4, 5 or 50 mg/ml.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In vitro* gene mutation study in bacteria**

		Official use only
	1. REFERENCE	
1.1 Reference	**A6.6.1/02** XXXX Escherichia coli reverse mutation assay (standard plate test and preincubation test) with BAS 350 I (Fipronil) 31 May 2005 (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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes OECD 471; 2000/32/EEC B.13/B.14; EPA 870.5100	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	**x**
3.1.1 Lot/Batch number	JML379A	
3.1.2 Specification	As given in section 2	**x**
3.1.2.1 Description	White powder	
3.1.2.2 Purity	98.9%	
3.1.2.3 Stability	Stable	
3.2 Study Type	Bacterial reverse mutation test	
3.2.1 Organism/cell type	E. coli: WP2 uvrA	
3.2.2 Deficiencies / Proficiencies	Deficient excision repair, tryptophan auxotroph (trp^-)	
3.2.3 Metabolic activation system	S-9 mix (derived from Aroclor 1254-induced rat liver)	
3.2.4 Positive control	With S-9 mix: 2-aminoanthracene (2-AA) Without S-9 mix: 4-nitroquinoline-N-oxide (4-NQO)	
3.3 Administration/ Exposure; Application of test substance		
3.3.1 Concentrations	0, 20, 100, 500, 2500, 5000 µg/plate (standard plate test); 0, 20, 100, 500, 2500, 5000 µg/plate (preincubation test)	
3.3.2 Way of application	Substance dissolved in Dimethyl sulfoxide (DMSO)	
3.3.3 Pre-incubation time	20 minutes (preincubation test only)	
3.3.4 Other modifications	None	
3.4 Examinations		

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In vitro* gene mutation study in bacteria**	
3.4.1 Number of cells evaluated	Not applicable	
4.1 Genotoxicity 4.4.1 Without metabolic activation 4.4.2 With metabolic activation **4.2 Cytotoxicity**	**4. RESULTS AND DISCUSSION** No evidence of mutagenicity No evidence of mutagenicity Yes: concentrations at and above 2500 µg/plate	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** In a supplementary reverse gene mutation assay, Fipronil was tested in E. coli WP2 uvrA at the concentration of 20, 100, 2500 and 5000 µg/plate using DMSO solvent in the presence and absence of S9 activation in two independent sets of experiments (standard plate test and preincubation test). The S9-mix was derived from male Sprague-Dawley rats. The rats were induced by a single i.p. injection of Aroclor 1254 (500 mg/kg) five days before sacrifice. The positive controls 4-nitroquinoline-N-oxide (4-NQO) and 2-aminoanthracene (2-AA) were used in the absence and presence of S9-mix, respectively. Plate incorporation assay: One-half (0.5) milliliter of S9 or Sham mix, 100 µL of tester strain and 100 µL of vehicle, positive control or test article were added to 2.0 ml of molten selective top agar. Within 30 seconds after mixing, the mixture was overlaid onto the surface of minimal agar plates. Plates were incubated for 48 to 72 hours at 37°C. Triplicate plates were prepared for each concentration condition. Likewise, triplicate plates with negative and respective positive control substances were prepared for each sub-experiment. Each plate was checked for precipitates and status of the background lawn and the revertant colonies were counted. Means and standard deviations were calculated from the mutation assay data. Pre-incubation assay: 0.1 ml test solution or vehicle, 0.1 ml bacterial suspension and 0.5 ml S-9 mix were incubated at 37°C for the duration of about 20 minutes using a shaker. Subsequently, 2 ml of soft agar was added and, after mixing, the samples were poured onto the agar plates within approx. 30 seconds. After incubation at 37°C for 48 – 72 hours in the dark, the bacterial colonies were counted.	**x** **x**

Section A6.6.1 **Annex Point IIA, VI.6.VI.6.1**	***In vitro* gene mutation study in bacteria**

		Evaluation criteria For a test article to be considered positive, it must produce a dose-related and reproducible increase in the number of revertant colonies, i.e. about doubling of the spontaneous mutation rate either without S-9 mix or after adding a metabolizing system. A test substance is generally considered non-mutagenic in this test if the number of revertants were within the historical negative control range under all experimental conditions in two experiments carried out independently of each other.
5.2	**Results and discussion**	(See Tables A6.6.1.2-1) Precipitates were observed at concentrations 500 µg/plate in both the plate-incorporation and the pre-incubation experiments. A slight decrease in the number of trp^+ revertants was observed depending on the test conditions at doses ≥ 2500 µg/plate. The positive controls yielded increases of revertant numbers in a range expected for the respective strains and thus demonstrated the sensitivity of the test system. There was no relevant elevation in the number of trp^+-revertants colonies in any of the tests. It was concluded that Fipronil was not mutagenic in E.coli strain WP2 uvrA, in the presence or absence of metabolic activation under the conditions of the study.
5.3	**Conclusion**	Under the experimental conditions chosen, it is concluded that Fipronil is not a mutagenic agent in a bacterial reverse mutation test using E. coli WP2 uvrA.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Table A6.6.1.2-1: Ames Test with Fipronil: Mean number of E. coli revertants

E. coli WP2 uvrA	**Plate incorporation assay**		**Pre-incubation assay**	
Metabol. Activation	**-S9**	**+S9**	**-S9**	**+S9**
Neg. control (DMSO)	37 ± 3	33 ± 3	30 ± 4	28 ± 4
Fipronil				
20 µg/plate	34 ± 3	33 ± 3	32 ± 3	26 ± 6
100 µg/plate	35 ± 5	33 ± 3	27 ± 5	20 ± 1
500 µg/plate	33 ± 4	28 ± 2	24 ± 4	24 ± 4
2500 µg/plate	26 ± 2	35 ± 2	24 ± 1	18 ± 5
5000 µg/plate	26 ± 2	27 ± 3	19 ± 3	18 ± 4
Pos. control				

E. coli WP2 uvrA		Plate incorporation assay		Pre-incubation assay	
Metabol. Activation		**-S9**	**+S9**	**-S9**	**+S9**
4-NQO µg/plate	5	651 ± 21	–	577 ± 16	–
2-AA µg/plate	60	–	208 ± 22	–	264 ± 37

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil: BAS 350 I 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.3.2 Way of application : *Dimethyl ~~sulfoxide~~* sulphoxide
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *at the concentration of* 0, *20, 100, 2500 and 5000 µg/plate* Benzo(a)pyrene was used to demonstrate the efficacy of the S-9 mix. *Plate incorporation assay: One-half (0.5) milliliter of S9 ~~or Sham~~ mix,*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in human lymphocytes

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.6.2/01** 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. XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not stated but compliant with OECD 473 and EU 92/69/EEC (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	none	
		3. MATERIALS AND METHODS	
3.1	**Test material**		X
3.1.1	Lot/Batch number	IGB 438	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	White crystalline solid	
3.1.2.2	Purity	95–97%	
3.1.2.3	Stability	Stable	
3.2	**Study Type**		
3.2.1	Organism/cell type	Human lymphocytes	
3.2.2	Deficiencies / Proficiencies	None	
3.2.3	Metabolic activation system	Yes – study conducted in the absence and presence of S9	
3.2.4	Positive control	Yes, methyl methanesulphonate and cyclophosphamide	
3.3	**Administration/ Exposure; Application of test substance**		
3.3.1	Concentrations	4.69, 9.38, 18.75, 37.5, 75, 150 and 300 µg/ml.	
3.3.2	Way of application	Dissolved in dimethyl sulfoxide	X
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	100 metaphases from each culture (where possible)	
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.4.1	Without metabolic activation	No	

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in human lymphocytes	
4.4.2 With metabolic activation **4.2 Cytotoxicity**	No The highest dose level (300 µg/ml) caused a reduction in mitotic index of 71 and 66% in the absence and presence of S9 mix respectively	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was assessed for its potential to induce chromosome aberrations *in vitro* in cultured human lymphocytes according to the following study design. Whole blood cultures were established in HEPES buffered RPMI medium containing 20% foetal calf serum, and incubated at 37°C in the presence of PHA for 44 hours before treatment. Two blood donors, one male and one female, were used to provide duplicate cultures. Fipronil, dissolved in dimethyl sulfoxide (DMSO), was added at 1% v/v to the cultures to give final concentrations of 4.69, 9.38, 18.75, 37.5, 75, 150 and 300 µg/ml. The highest dose level was at the limit of solubility in the culture medium. One set of cultures was treated in the absence of S9 mix and one set in its presence. The final concentration of S9 in the culture medium was 2% v/v. Positive control cultures were treated with methyl methanesulphonate in the absence of S9 mix (final concentrations: 50, 75 and 100 µg/ml) and cyclophosphamide in its presence (final concentrations: 12.5, 25 and 50 µg/ml). Duplicate solvent control cultures were also established. After three hours of treatment the medium was removed from the cultures and replaced with fresh medium. Cultures were then incubated for a further 25 hours before harvesting. One hour prior to harvest, colchicine was added at a final concentration of 1 µg/ml to arrest dividing cells in metaphase. Cells were harvested using 0.075M KCl as a hypotonic treatment to cause swelling, and then fixed in methanol/glacial acetic acid. Slides were prepared and stained with Giemsa. The slides were examined for mitotic index in order to assess toxicity. Three dose levels were then selected for the metaphase analysis of which the highest caused a 50 – 80% reduction in mitotic index compared with the solvent control. Slides were analysed under code. One hundred metaphase cells from each culture were analysed for chromosome aberrations.	**x** **x**

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in human lymphocytes

5.2	**Results and discussion**	(See Table A6.6.2.1-1) The dose levels of Fipronil selected for the metaphase analysis under both metabolic activation conditions were 75, 150 and 300 µg/ml. The highest, 300 µg/ml caused a reduction in mitotic index of 71% and 66% in the absence and the presence of S9 mix, respectively. No statistically significant increases in the number of aberrations were observed in cultures treated with Fipronil when compared with the solvent control. All values fell within the historical control range. Both positive control compounds caused large significant increases in aberrations.	X
5.3	**Conclusion**	Fipronil did not induce chromosome aberrations in human lymphocytes when tested to its limit of toxicity and solubility in the absence and presence of S9 mix.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.2.1-1 Results of in-vitro chromosome aberration assay in human lymphocytes

Group	Dose level [µg/ml]	No. cells scored	Number of aberrations						% aberrant cells		Mitotic index
			Chromatid		Chromosome		Others #	Gaps	incl. gaps	excl. gaps	
			ctb	cte	csb	cse					
Without S9-mix											
Vehicle control (DMSO)	0	200	1	0	1	0	4	3	4.5	3.0	3.4
Fipronil	75	200	1	0	1	0	1	4	2.5	1.5	3.4
	150	200	0	0	1	0	1	5	3.5	1.0	3.1
	300	183	1	0	2	0	0	2	2.7	1.6	1.0
Positive control (MMS)	100	75	10	8	6	0	0	6	28***	23***	N.D.
With S9-mix											
Vehicle control (DMSO)	0	200	2	0	1	1	1	4	4.0	2.0	3.4
Fipronil	75	200	0	0	0	0	1	6	3.5	0.5	2.2
	150	186	0	0	1	0	0	4	2.7	0.5	2.2
	300	162	0	0	0	0	~~0~~ 1	5	3.1	0.6	1.2
Positive control (CPA)	25	67	28	7	10	0	0	8	39***	30***	N.D.

Statistical evaluation: *** = p<0.001 (Chi-square analysis)
#: numerical aberrations (~~*endoduplicated*~~ endoreduplicated, hyperdiploid, polyploid)
MMS = Methyl methanesulfonate; CPA = Cyclophosphamide
ctb = chromatid break; cte = chromatid exchange; csb = chromosome break; cse = chromosome exchange

X

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *Microtest Research Limited;* UK 3.1 Test material: Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.3.2 Way of application/5.1 Materials and methods: *Dimethyl* ~~*sulfoxide*~~ sulphoxide to give 30 mg/ml
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *HEPES buffered RPMI medium containing 20% foetal calf serum, and* 50 µg/ml gentamycin, *incubated at 37°C in the presence of* Phytohaemagglutinin (*PHA*) *for 44 hours before treatment.* *Slides were prepared and stained with* Gurr's *Giemsa* R66, to observe them with a microscope. 5.2 Results and discussion: *The dose levels of Fipronil selected for the metaphase analysis* ~~*under both metabolic activation conditions*~~ without and with S-9 *were 75, 150 and 300 µg/ml.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in Chinese hamster cells

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.6.2/02** XXXX Fipronil: Chromosome aberration test in CHL cells *in vitro*. (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes – Compliant with JMAFF 4200; not stated but compliant with OECD 473 and EU 92/69/EEC (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	TAK 1747	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	White powder	
3.1.2.2	Purity	98.3 % w/w	
3.1.2.3	Stability	Stable	X
3.2	**Study Type**		
3.2.1	Organism/cell type	Chinese hamster lung cells	
3.2.2	Deficiencies / Proficiencies	Not applicable	
3.2.3	Metabolic activation system	Yes – study conducted in the absence and presence of S9	
3.2.4	Positive control	Yes, Mitomycin C and Cyclophosphamide	
3.3	**Administration/ Exposure; Application of test substance**		
3.3.1	Concentrations	<u>Main study, 6-h treatment without S9 mix:</u> 7.5, 15, 30, 45 and 60 µg/ml. <u>Main study, 6-h treatment with S9 mix:</u> 7.5, 15, 30, 60 and 120 µg/ml. <u>Main study, 24-h treatment without S9 mix:</u> 3.75, 7.5, 15, 30 and 60 µg/ml. <u>Main study, 48-h treatment without S9 mix:</u> 3.75, 7.5, 15, 22.5 and 30 µg/ml	
3.3.2	Way of application	Dissolved in dimethyl sulfoxide (DMSO)	X
3.3.3	Pre-incubation time	24 hours	X
3.3.4	Other modifications	None	
3.4	**Examinations**		

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in Chinese hamster cells	
3.4.1 Number of cells evaluated	100 metaphases from each culture, three dose levels evaluated	
4.1 Genotoxicity 4.4.1 Without metabolic activation 4.4.2 With metabolic activation **4.2 Cytotoxicity**	**4. RESULTS AND DISCUSSION** Increased incidence of aberrations at cytotoxic dose levels after 6-h treatment. No evidence at non-cytotoxic concentrations and after 24-h or 48-h treatment. Increased incidence of aberrations in the presence of cytotoxicity after 6-h treatment. Increase of cytotoxicity with steep concentration-response curve. After 6-h treatment, cell counts were 84% at 31.25 µg/ml and 24% at 62.5 µg/ml without S9 mix and 87% and 51% at the respective concentrations with S9 mix.	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was assessed for its potential to induce chromosome aberrations *in vitro* in cultured Chinese hamster lung (CHL) cells according to the following study design. CHL cultures were established in MEM medium containing 10% foetal calf serum, and incubated at 37°C and 5% CO_2 for approximately 24 hours prior to treatment. A preliminary cytotoxicity test was performed using 24 and 48 hours of continuous exposure without metabolic activation. A 6-hour exposure period both with and without metabolic activation followed by an 18-hour recovery period in treatment-free media was also included. The final concentrations of Fipronil (dissolved in Dimethyl sulfoxide, DMSO) in the culture medium were 3.9, 7.81, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 µg/ml. The highest dose level was limited by precipitation. Duplicate cultures were used for each treatment including the solvent control. The final concentration of S9 mix in the culture medium was 1% v/v. Two hours prior to harvest, a final concentration of 0.1 µg/ml of Colcemid (colchicine) was added to arrest dividing cells in metaphase. Cells were harvested by trypsinisation, 0.075M KCl was used as a hypotonic treatment to cause swelling, and then the cultures were fixed in methanol/glacial acetic acid. Slides were prepared and stained with Giemsa. Growth inhibition was assessed by cell counts and results used to select dose levels for the main study.	x x

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in Chinese hamster cells	
5.2	**Results and discussion**	In the main study, four treatment regimes were used as in the preliminary toxicity test. For the 6-hour treatment, final concentrations of Fipronil were 7.5, 15, 30, 45 and 60 µg/ml in the absence of S9 mix, and 7.5, 15, 30, 60 and 120 µg/ml in the presence of S9 mix. For the 24-hour treatment without S9 mix, final concentrations were 3.75, 7.5, 15, 30 and 60 µg/ml. For the 48-hour treatment without S9 mix, final concentrations used were 3.75, 7.5, 15, 22.5 and 30 µg/ml. Positive control cultures used 0.05 µg/ml of Mitomycin C for the 24- and 48-hour treatments without S9 mix, and 10 µg/ml of Cyclophosphamide for the 6-hour treatments. Solvent control cultures were also included. All cultures were treated in duplicate. Three dose levels from each treatment were then selected for the metaphase analysis. Slides were analysed under code and one hundred metaphase cells from each culture were scored for chromosome aberrations. (See Tables A6.6.2.2-1 and -2) In the preliminary toxicity test, Fipronil caused a dose-related increase in cell toxicity. At 250 µg/ml and above, a precipitate was observed at both dosing and harvesting. The cell counts from the main chromosome aberration test showed similar toxicity to that in the preliminary test. Dose levels selected for the metaphase chromosome analysis were: 30, 45 and 60 µg/ml for the 6-hour treatment without S9 mix, 15, 30 and 60 µg/ml for the 6-hour treatment with S9 mix, 7.5, 15 and 30 µg/ml for the 24-hour treatment without S9 mix, and 7.5, 15 and 22.5 µg/ml for the 48-hour treatment without S9 mix. At the highest dose level for each treatment set, the cell count relative to the control was 44%, 72%, 47% and 46%, respectively. For the test with S9 mix, the higher dose level of 120 µg/ml caused a reduction in cell count to 29% of the solvent control value but there were no scorable metaphase cells. In both of the 6-hour treatments, Fipronil induced significant increases in the proportion of aberrant cells in cultures treated with the toxic highest dose level, 60 µg/ml. No significant increases in the number of aberrant cells were observed at lower dose levels or in cultures treated for 24- or 48-hours.	**x** **x**
5.3	**Conclusion**	Toxic dose levels of Fipronil caused clastogenicity in Chinese hamster lung cells in both the absence and presence of metabolic activation following 6-hours of exposure. No clastogenic effects were observed at lower dose levels or in cultures treated for 24- or 48-hours.	**x**

Section A6.6.2 **Annex Point IIA, VI.6.VI.6.2**	***In vitro* cytogenicity study in mammalian cells** – Chromosome aberration study in Chinese hamster cells

5.3.1 Reliability	1
5.3.2 Deficiencies	None

Table A6.6.2.2-1 Summary of chromosome aberration frequencies – 6 hour treatments with and without S9 mix

Group	Dose level (µg/ml)	No. of aberrations						No. of aberrant cells			
		Chromatid		Chromosome		Others#	Total gaps	Excluding gaps	Mean %	Including gaps	Mean %
		ctb	cte	csb	cse						
Without S9-mix											
Vehicle control (DMSO)	-	0 0	0 0	0 3	0 0	0 0	1 0	0 1	0.5	1 1	1.0
Fipronil	30	0 0	0 0	0 1	0 0	0 0	0 0	0 1	0.5	0 1	0.5
	45	0 1	0 5	0 1	1 1	0 0	0 1	1 6	3.5	1 6	3.5*
	60	9 13	20 19	0 0	0 1	0 1	0 4	14 15	14.5***	14 16	15.0***
Positive control (CP)	10	1 0	0 0	0 1	0 0	0 0	2 0	1 1	1.0	3 1	2.0
With S9-mix											
Vehicle control (DMSO)	-	0 0	0 1	1 0	2 0	0 0	1 0	3 1	2.0	4 1	2.5
Fipronil	15	0 0	0 0	0 0	0 1	0 0	1 0	0 1	0.5	1 1	1.0
	30	0 0	0 0	1 0	0 1	0 0	3 1	1 1	1.0	4 2	3.0
	60	3 2	6 4	0 0	0 3	0 0	2 2	6 5	5.5	8 5	6.5*
Positive control (CP)	10	24 22	31 30	6 4	0 1	0 1	11 16	31 31	62.0***	33 35	68.0***

Statistical evaluation: * $p < 0.05$ *** $p < 0.001$
#: >10 aberrations/cell (not included in total aberrations); CP: Cyclophosphamide
ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange

Table A6.6.2.2-2 Summary of metaphase analysis data – 24 and 48 hour treatments without S9 mix

Group	Dose level (µg/ml)	No. of aberrations						No. of aberrant cells			
		Chromatid		Chromosome		Others #	Total gaps	Excluding gaps	Mean %	Including gaps	Mean %
		ctb	cte	csb	cse						
24-hours without S9-mix											
Vehicle control (DMSO)	-	0 0	0 0	0 0	1 0	0 0	0 0	1 0	0.5	1 0	0.5
Fipronil	7.5	0 0	0 0	1 0	0 1	0 0	0 1	1 1	1.0	1 2	1.5
	15	0 0	0 0	0 0	0 1	0 0	1 1	0 1	0.5	1 2	1.5
	30	1 0	0 0	0 1	1 0	0 0	0 0	2 1	1.5	2 1	1.5
Positive control (MMC)	0.05	8 7	12 19	2 1	0 0	0 0	6 2	17 27	29.3***	22 29	34.0***
48-hours without S9-mix											
Vehicle control (DMSO)	-	0 0	0 0	1 0	0 1	0 0	0 0	1 1	1.0	1 1	1.0
Fipronil	7.5	1 0	0 0	2 0	1 1	0 0	0 1	3 1	2.0	3 2	2.5
	15	0 0	0 0	0 1	0 0	0 0	0 0	0 1	0.5	0 1	0.5
	22.5	0 0	0 0	0 0	0 0	1 0	2 2	1 0	0.5	3 2	2.5
Positive control (MMC)	0.05	16 20	23 25	5 11	3 1	2 1	3 1	27 32	59.0***	27 33	60.0***

Statistical evaluation: *** $p < 0.001$
#: >10 aberrations/cell (not included in total aberrations); MMC = Mitomycin C
ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.2 Reference: ~~*5 April 1995*~~ XXXX 3.1 Test material: Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.3.2 Way of application/5.1 Materials and methods: *Dimethyl* ~~*sulfoxide*~~ sulphoxide to give 30 mg/ml 3.3.3 Pre-incubation time: ~~*24 hours*~~
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *and incubated at 37°C and 5% CO_2* ~~*for approximately 24 hours prior to treatment.*~~ ~~*The final concentration of S9 mix in the culture medium was 1% v/v.*~~ *Colcemid* ~~*(colchicine)*~~ (demecolcine) *Slides were prepared and stained with* Gurr's *Giemsa* R66, to observe them with a microscope. 5.2 Results and discussion: There were no scorable metaphases at and above 125 µg/ml in the 6-hour treatment without S9, at 1000 µg/ml in the 6-hour treatment with S9, at and above 62.5 µg/ml in the 24-hour treatment, or at and above 31.25 µg/ml in the 48-hour treatment group. *At the highest dose level for each treatment set* selected for the metaphase analysis*, the cell count relative to the control was 44%, 72%, 47% and 46%, respectively.* 5.3 Conclusion: Fipronil was shown to be highly toxic to CHL cells in vitro in all four treatment cases, with a very steep dose-response curve. *No clastogenic effects were observed at lower dose levels or in cultures treated for 24- or 48-hours* at no cytotoxic dose levels.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.3 **Annex Point IIA, VI.6.VI.6.3**	***In vitro* gene mutation assay in mammalian cells**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.6.3/01** XXXX M&B 46030: Investigation of mutagenic activity at the HGPRT locus in a Chinese hamster V79 cell mutation system – Amended final report. XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes: OECD 476 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**		X
3.1.1	Lot/Batch number	JJW2092/1	
3.1.2	Specification	As given in section 2	X
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	97.2%	
3.1.2.3	Stability	Stable	X
3.2	**Study Type**		
3.2.1	Organism/cell type	Chinese hamster (V79) cells	
3.2.2	Deficiencies / Proficiencies	None (normal HPRT-proficient cells)	
3.2.3	Metabolic activation system	With and without (S9) mix	
3.2.4	Positive control	Without S9-mix: Ethyl methansulphonate (EMS): 1000 µg/ml With S9-mix: Dimethylbenzanthracene (DMBA): 10 µg/ml	X
3.3	**Administration/ Exposure; Application of test substance**		
3.3.1	Concentrations	0 – 1000 µg/ml (precipitation occurred above this level)	X
3.3.2	Way of application	Dilution of test substance and positive controls in dimethyl sulfoxide (DMSO)	X
3.3.3	Pre-incubation time	None	
3.3.4	Other modifications	None	
3.4	**Examinations**		
3.4.1	Number of cells evaluated	Triplicates each of two cultures for each test concentration with or without S9-mix, roughly 200 colonies/plate (range 91–269/plate)	X
		4. RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		

Section A6.6.3 **Annex Point IIA, VI.6.VI.6.3**	***In vitro* gene mutation assay in mammalian cells**	
4.4.1 Without metabolic activation	No	
4.4.2 With metabolic activation	No	
4.2 Cytotoxicity	Preliminary toxicity test No evidence of toxicity was observed in the Fipronil-treated cultures. Therefore, the concentrations selected for use in the main mutation assays in the absence and presence of S9 mix were 0.8, 4, 20, 100 and 500 µg/ml. Mutation assays In the absence of S9 mix, there was no evidence of toxicity at any dose level of Fipronil in either mutation assay. In the presence of S9 mix, there was no evidence of toxicity (relative to solvent control) in any Fipronil-treated culture in the first mutation assay. However, a slight reduction in survival was seen in the second assay at 100 and 500 µg/ml of Fipronil (47.1% and 62.7% of the solvent control value, respectively).	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Following a preliminary toxicity test, two independent mutation assays were conducted with cultures of Chinese hamster (V79) cells exposed to Fipronil in the absence and presence of a metabolic activating system (S9 mix). Forward mutation rates at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene locus were evaluated as resistance to the base analogue 6 thioguanine (6-TG), which kills HPRT-proficient cells. In the preliminary toxicity test, cells were exposed for 3 hours to concentrations between 0.8 and 500 µg/ml of Fipronil in the presence and absence of S-9 mix. The highest concentration was limited solubility of the test material in aqueous medium since extreme precipitation occurred on addition of dose levels of > 500 µg/ml to the culture medium. At the end of the exposure period, the cells were washed and seeded in non-selective medium to assess toxicity. The plating efficiency was determined by colony counts.	

Section A6.6.3 **Annex Point IIA, VI.6.VI.6.3**	***In vitro* gene mutation assay in mammalian cells**	
	In both mutation assays, 7.5 x 10^5 cells were seeded approximately 24 hours prior to treatment. They were treated with a range of concentrations of Fipronil, dissolved in DMSO, and either serum-free medium in the absence of metabolic activation, or S9 mix in the presence of activation. Vehicle (DMSO) control and positive controls (ethyl methanesulphonate, EMS: 1000 µg/ml in the absence of metabolic activation and dimethylbenzanthracene, DMBA: 10 µg/ml in both the presence and absence of S9 mix) were included. Duplicate cultures were used for all treatments. Dose levels of Fipronil used in the mutation tests were 0.8, 4, 20, 100, 500 and 1000 µg/ml without S9 mix and 0.8, 4, 20, 100 and 500 µg/ml with S9 mix. Residual volumes of 20 and 100 µg/ml of Fipronil prepared for the non-activated cultures were analysed to confirm the achieved test material concentration. Results showed that adequate amounts (80 and 100% of nominal) were achieved. Variations were considered to be due to the compound's insolubility in water. Cultures were gassed with 5% CO_2 in air and incubated for three hours at 37°C. At the end of this treatment-period, the cells were washed three times with HBSS and survival determined. The cultures were passaged, gassed with 5% CO_2 in air and re-incubated at 37°C the cultured and re-passaged as necessary during a 7-day expression period. At the end of the expression period, the cells were plated for survival and 6-TG resistance. The cultures were trypsinised, resuspended in DMEM containing 10% foetal calf serum, and aliquots of cells (10^5 cells) seeded on to three 90 mm plates for the selection of 6-TG mutants. For plating efficiency, cell suspensions (200 cells) were seeded on three plates. After 2-3 hours, 6-TG (10 µg/ml) was added to the 6-TG selection plates. Both sets of plates were incubated at 37°C in a 5% CO_2 atmosphere and 100% relative humidity for 6 days. At the end of this period, the colonies were fixed in methanol and stained with 10% Giemsa. The plates were scored by eye and the mutation frequency was calculated.	x
5.2 Results and discussion	(See Table A6.6.3.1-1) In the absence of S9 mix, plating efficiencies in Fipronil-treated cultures ranged from 72.9% to 132.9% (first assay) and from 56.7% to 93.0% (second assay) with no evidence of dose-related toxicity. No significant increases in mutant frequencies or in mutant colony numbers were seen in either assay. In the presence of S9 mix, plating efficiencies in treated cultures ranged from 74.7% to 106.7% (first assay) and from 64.0% to 111.5% (second assay) with no evidence of dose-related toxicity. Again, there were no significant increases in mutant frequencies or in mutant colony numbers in either assay.	x

Section A6.6.3 Annex Point IIA, VI.6.VI.6.3	***In vitro* gene mutation assay in mammalian cells**	

	The positive controls induced expected large increases in both the mutation frequencies and mutant colony numbers thereby demonstrating the efficacy of the S9 mix and the sensitivity of the assays.	X
5.3 Conclusion	Fipronil showed no potential to induce forward gene mutation in Chinese hamster V79 cells cultured in vitro.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	none	

X

Table A6.6.3.1-1 ~~**Summary of metaphase analysis data - 24 and 48 hour treatments without S9 mix**~~ **Main mutation assays - treatment means in the absence and in the presence of S-9 mix**

	Fipronil						**EMS**	**DMBA**
(µg/ml)	**0**	**0.8**	**4**	**20**	**100**	**500**	**1000**	**10**
1st mutation assay without S9 mix								
Plating efficiency	107.0	121.4	89.2	127.0	78.4	96.1	83.6	109.2
Mutation frequency [a]	0.3	0.6	7.5	1.3	0.0	2.9	136.0	3.7
2nd mutation assay without S9 mix								
Plating efficiency	89.5	72.5	78.0	63.0	84.3	92.0	73.2	81.5
Mutation frequency [a]	0.0	1.1	0.0	0.0	2.4	0.6	116.0	0.0
1st mutation assay with S9 mix								
Plating efficiency	97.7*	95.0	99.5	75.9	92.8	93.0	N.A	54.1
Mutation frequency [a]	5.4*	0.0	2.8	2.0	0.0	0.0	N.A	101.3
2nd mutation assay with S9 mix								
Plating efficiency	64.5	108.1	70.5	107.1	91.2	85.6	N.A	96.1
Mutation frequency [a]	0.6	1.5	0.0	1.1	0.0	0.0	N.A	49.7

[a] per 100,000 survivors;
* data from one culture only; remaining culture died due to cracked culture flask

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: XXXX 3.1 Test material: Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.2.4 Positive control: ~~*Ethyl methansulphonate*~~ Ethyl methanesulphonate 7, 12-*Dimethylbenzanthracene* 3.3.1 Concentration: ~~*0 – 1000 µg/ml (precipitation occurred above this level)*~~ 0.8, 4, 20, 100 and 500 µg/ml were the concentrations selected for the main study. 3.3.2 Way of application/5.1 Materials and methods: *Dimethyl ~~sulfoxide~~* sulphoxide to give 30 mg/ml 3.4.1 Number of cells evaluated: *Triplicates each of two cultures for each test concentration with or without S9-mix, roughly 200 colonies/plate ~~(range 91 – 269/plate)~~* for the plating efficiency and mutation frequency was evaluated per 10^5 surviving cells.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Residual volumes ~~of 20 and 100 µg/ml~~ of Fipronil prepared for the non-activated cultures were analysed to confirm the achieved test material concentration. ~~Results showed that adequate amounts (80 and 100% of nominal) were achieved. Variations were considered to be due to the compound's insolubility in water~~*. Information not found in the study report. 5.2 Results and discussion: Preliminary test: Some evidence of precipitation was observed at 100 µg/ml with increased precipitation at 500 µg/ml, in the absence and presence of S-9 mix. The suspensions formed were apparently homogeneous following brief vortex mixing. There was no evidence of dose-related toxicity in either the absence or presence of S-9 mix. The concentrations of fipronil selected for use in the main mutation assay were: 0.8, 4, 20, 100 and 500 µg/ml with and without S-9 mix. Toxicity tests: No evidence of toxicity was observed in non-activated cultures treated with fipronil at any level tested in either of two mutations assays, as determined by plating efficiency. In the presence of S-9 mix, no evidence of toxicity was observed in any treated culture in the first mutation assay, but there was a slight reduction in survival in cultures exposed to fipronil at 100 and 500 µg/ml (to 47.1% and 62.7% of the solvent control value respectively) in the second mutation assay.

	~~*No significant increases in mutant frequencies or in mutant colony numbers were seen in either assay.*~~ In the first mutation assay, only one culture (exposed at 4 µg/ml) gave a real increase in mutation frequency (11.6 per 10^5 survivors) and in mutant colony numbers, compared to the solvent control values of 0.0 and 0.6 per 10^5 survivors. Small increases in mutant colony over solvent control values were observed in other treated cultures, but these were neither consistent nor dose-related. In the second mutation assay, there were no real increases either in mutation frequencies or in mutant colony numbers, over the solvent control values, in any treated culture. *The positive controls induced expected large increases in both the mutation frequencies* (EMS: mean mutation frequency of 136.0 compared to control value of 0.3 in the first assay and 116.0 compared to 0 in the second assay. DMBA: 101.3 and 49.7 in the first and the second assays respectively, compared to 5.4 and 0.6.) *and mutant colony numbers thereby demonstrating the efficacy of the S9 mix and the sensitivity of the assays.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.4	***In vivo* genotoxicity study (1st micronucleus test)**
Annex Point IIA, VI.6.VI.6.4	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.6.4/01** XXXX M&B 46030: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes – EPA 84/1	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	JJW2092/1	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	97.2% w/w	
3.1.2.3	Stability	Stable	**X**
3.1.2.4	Maximum tolerable dose	25 mg/kg bw	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	4 – 6 weeks	**X**
3.2.6	Number of animals per group	Range-finding test: 2/sex/dose Main test: 5/sex/dose and sampling time	
3.2.7	Controls animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable (single application)	
3.3.3	Postexposure period	24, 48 or 72 hours after treatment	
3.3.4	Type	Gavage	
3.3.5	Concentration	0, 1, 5 or 25 mg/kg bw	
3.3.6	Vehicle	0.5% aqueous methyl cellulose	
3.3.7	Concentration in vehicle	Main test: 0.1, 0.5, and 2.5 mg Fipronil/ml	
3.3.8	Total volume applied	10 ml/kg bw	
3.3.9	Controls	Vehicle	
3.3.14	Substance used as Positive Control	Chlorambucil	

Section A6.6.4	***In vivo* genotoxicity study (1st micronucleus test)**
Annex Point IIA, VI.6.VI.6.4	

3.4	**Examinations**		
3.4.1	Clinical signs	Yes	
3.4.2	Tissue	Yes Bone marrow	
		Number of animals:	all animals
		Number of cells:	At least 2000 erythrocytes/animal
		Time points:	24, 48, 72 h after treatment
		Type of cells	erythrocytes in bone marrow
		Parameters:	Frequency of micronuclei in polychromatic erythrocytes polychromatic/normochromatic erythrocytes ratio
3.5	**Further remarks**	Body weights were determined prior to treatment and again immediately before termination	
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	Range-finding test: Mortality, convulsions, increased motor activity at 100 and 200 mg/kg bw. 2 of 4 mice administered 25 mg/kg bw showed piloerection. No clinical signs in the main study (dose levels tested up to 25 mg/kg bw)	
4.2	**Haematology / Tissue examination**	The ratio of polychromatic to normochromatic (mature) erythrocytes was similar to control values. Therefore there was no evidence of bone marrow toxicity.	
4.3	**Genotoxicity**	No	
4.4	**Other**	A loss of bodyweight was noted 24 hours post-treatment in eight of the ten animals treated with 25 mg/kg bw. At this same time point, nine of the ten positive control animals given 30 mg/kg bw of chlorambucil lost body weight.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil was assessed for its potential to cause chromosome damage (clastogenicity) *in vivo* by quantification of micronuclei in bone marrow erythrocytes following single oral administration to mice. In a preliminary toxicity test, groups of two male and two female CD-1 mice were given a single oral dose by gavage of either 25, 50, 100 or 200 mg/kg bw of Fipronil in aqueous Methylcellulose (0.5%) at the dose volume of 10 ml/kg bw. Based on the results of this study, in the micronucleus study groups of 5 male and 5 female mice were given a single oral gavage dose of either 0, 1, 5 or 25 mg/kg bw of Fipronil. The controls (0 mg/kg) received the vehicle alone. A similar sized group was given 30 mg/kg bw of Chlorambucil in aqueous ethanol (10%) as the positive control. Dose preparations were analysed for the achieved concentration of the test material. Animals were observed daily for clinical signs of toxicity. Individual body weights were recorded prior to dosing and at termination. Five males and five females from each group were killed 24 hours after treatment. Additional groups of five males and five females from the vehicle control and 25 mg/kg bw of Fipronil groups were killed 48 and 72 hours after treatment. Bone marrow smears were made from each animal. A total of at least 2000 erythrocytes per animal were examined for the presence of micronuclei, using the light microscope. The frequencies	

Section A6.6.4	***In vivo* genotoxicity study (1st micronucleus test)**
Annex Point IIA, VI.6.VI.6.4	

		of micronucleated cells per 1000 polychromatic erythrocytes were analysed statistically. The ratio of polychromatic cells to mature normochromatic cells was also calculated per each animal. The mice weighed between 18 and 25 g and were about 4-5 weeks old on arrival. They were acclimatised for at least 4 days prior to dosing and were housed by sex in groups of 2 (preliminary toxicity test) or 5 (micronucleus test).	
5.2	**Results and discussion**	<u>Dose preparation analysis</u> Results of dose preparation analysis of samples from the preliminary toxicity test were within the range of 88 to 104% of nominal. Values for all dose levels from the main micronucleus test were between 80 and 108% of nominal. Preliminary toxicity test In the preliminary toxicity test, two mice were found dead approximately 18 hours after dosing, one from each of 100 and 200 mg/kg bw groups. The other animals at these dose levels were killed *in extremis* approximately 18 hours post-dosing showing convulsions and increased motor activity. At 25 mg/kg bw, two mice exhibited piloerection post-dosing. All animals given 25 and 50 mg/kg bw survived to termination. All mice given 50 mg/kg bw and half of those dosed with 25 mg/kg bw lost weight during the first 24 hours after dosing. Because bone marrow proliferation was depressed at 50 mg/kg, the highest dose level selected for main micronucleus test was 25 mg/kg bw. Micronucleus test (See Table A6.6.4.1-1) No clinical signs were seen. A loss of body weight was noted 24 hours post-treatment in eight of the ten animals treated with 25 mg/kg bw. At this same time point, nine of the ten positive control animals given 30 mg/kg bw of chlorambucil lost body weight. The frequencies of micronucleated polychromatic erythrocytes in animals killed 24, 48 or 72 hours after treatment with Fipronil were similar to those in vehicle controls. Furthermore, the ratio of polychromatic to normochromatic erythrocytes in the test material-treated groups was similar to vehicle controls. The positive control, Chlorambucil, caused a significantly higher frequency of micronucleated polychromatic cells which demonstrated the sensitivity of the system. The ratio of polychromatic to mature erythrocytes was reduced by Chlorambucil indicating toxicity to the bone marrow cells.	**x** **x**
5.3	**Conclusion**	Fipronil showed no potential to induce chromosome damage (clastogenicity as evidenced by micronuclei formation) and no bone marrow toxicity in mice following acute oral administration of up to 25 mg/kg bw.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Table A6.6.4.1-1 Summary micronucleus test evaluation

Exposure period	Treatment (mg/kg bw)		Sex	Micronucleated polychromatic cells / 1000		P : N
				Mean ± SD	Range	
24 h	Fipronil	0	M+F	0.3 ± 0.5	0.0 – 1.0	0.9
		1	M+F	0.7 ± 0.7	0.0 – 2.0	0.9
		5	M+F	0.4 ± 0.7	0.0 – 1.9	0.8
		25	M+F	0.5 ± 0.7	0.0 – 2.0	0.9
	Chlorambucil	30	M+F	67.6 ± 22.9**	39.8 – 110.2	0.5
48 h	Fipronil	0	M+F	1.0 ± 1.0	0.0 – 2.9	0.9
		25	M+F	0.6 ± 0.8	0.0 – 1.9	0.9
72 h	Fipronil	0	M+F	0.6 ± 0.7	0.0 – 2.0	0.8
		25	M+F	1.0 ± 0.7	0.0 – 1.9	0.9

P:M = Ratio of polychromatic : normochromatic (=mature) cells
Statistical evaluation: ** = p< 0.01 (Mann-Whitney U-test, one-tailed)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.2.5 Age/weight at study initiation: males: 23.9-29.7 g, females: 19.7-24.2 g 3.5 Further remarks: ~~*Body weights were determined prior to treatment and again immediately before termination*~~ All animals were weighed on the day of treatment and again immediately before termination. In addition, the animals in the preliminary toxicity test were weighed immediately prior to dosing and daily thereafter until termination.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *Because bone marrow proliferation was depressed at* 25 and *50 mg/kg* giving a mean ratio of 0.5 in each case compared to an historical mean value for vehicle control animals of approximately 0.9; *the highest dose level selected for main micronucleus test was 25 mg/kg bw.* 50 mg/kg was the highest dose level tolerated by the mice in this study: The mean frequency of micronucleated polychromatic erythrocytes observed at 50 mg/kg was 0.5, with a range of 0.0-2.0, compared with a mean of 0.6 with a range of 0.0-2.0 observed in vehicle control after 72 hours. The ratio at the same dose was 0.6 and showed a reduction compared to the corresponding vehicle control value of 0.8.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.4 **Annex Point IIA, VI.6.VI.6.4**	***In vivo* genotoxicity study (2nd micronucleus test)**	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.6.4/02** XXXX M&B 46030: Mouse micronucleus test to comply with OECD Guideline 474 (1983) (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD 474 (1983); EPA Guideline 84-1 (1987); EEC Annex V Method B.12 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**x**
3.1.1	Lot/Batch number	DA 832	
3.1.2	Specification	As given in Section 2	**x**
3.1.2.1	Description	Fine cream white powder	
3.1.2.2	Purity	96.2% w/w	
3.1.2.3	Stability	Stable	**x**
3.1.2.4	Maximum tolerable dose	50 mg/kg bw	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD 1	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	5 – 6 weeks males 23.5 – 27.3 g females 18.8 – 23.1 g	**x**
3.2.6	Number of animals per group	10 (5 male and 5 female)	
3.2.7	Controls animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable (single application)	
3.3.3	Postexposure period	24, 48 and 72 hours after treatment	
3.3.4	Type	Gavage	
3.3.5	Concentration	Main test: 12.5, 25 or 50 mg/kg bw	
3.3.6	Vehicle	0.5% aqueous solution of carboxymethyl cellulose	**x**

Section A6.6.4 **Annex Point IIA, VI.6.VI.6.4**		***In vivo* genotoxicity study (2nd micronucleus test)**	
3.3.7	Concentration in vehicle	Main test: 0.125, 2.5, or 5 mg/ml	x
3.3.8	Total volume applied	10 ml/kg body weight	
3.3.9	Controls	Negative control: Vehicle	
3.3.14	Substance used as Positive Control	Chlorambucil	
3.4	**Examinations**		
3.4.1	Clinical signs	Yes – daily	
3.4.2	Tissue	Yes Bone marrow	
		Number of animals: all animals Number of cells: At least 2000 erythrocytes/animal Time points: 24, 48, 72 h after treatment Type of cells erythrocytes in bone marrow Parameters: Frequency of micronuclei in polychromatic erythrocytes polychromatic/normochromatic erythrocytes ratio	
3.5	**Further remarks**	Body weights were determined prior to treatment and again immediately before termination	x
		4. RESULTS AND DISCUSSION	
4.1	**Clinical signs**	In the main micronucleus test, eight of the thirty mice treated with 50 mg/kg of fipronil showed clinical signs of toxicity including hunched posture (8 mice), piloerection (5), convulsions (4), underactivity (3) and slow respiration (1). No clinical signs were seen at 12.5 and 35 mg/kg. One female given Chlorambucil as the positive showed hunched posture and underactivity 3 hours post-dosing.	
4.2	**Haematology / Tissue examination**	No real evidence of toxicity of Fipronil to the bone marrow (as evidenced by depression of bone marrow proliferation, i.e. reduced ratio of polychromatic to mature erythrocytes) in the preliminary range-finding test at dose levels causing clinical signs of neurotoxicity and lethality. No evidence of bone marrow toxicity in the main assay at dose levels of up to 50 mg/kg bw.	
4.3	**Genotoxicity**	No	
4.4	**Other**	Most of the fipronil high dose level mice lost weight after dosing. Body weight was unaffected at 12.5 and 25 mg/kg bw.	
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Fipronil was assessed for its potential to cause chromosome damage (clastogenicity) *in vivo* by quantification of micronuclei in bone marrow erythrocytes following single oral administration to male and female CD-1 mice. The mice used weighed between 16 and 23 g and were about 4-5 weeks old on arrival. They were acclimatised for at least 4 days prior to dosing and were housed by sex in groups of 2 (preliminary test) or 5 (micronucleus test).	

Section A6.6.4 **Annex Point IIA, VI.6.VI.6.4**		***In vivo* genotoxicity study (2nd micronucleus test)**	
		In a preliminary toxicity test, groups of two male and two female CD-1 mice were given a single oral dose by gavage of either 30, 50, 70 or 120 mg/kg bw of Fipronil suspended in 0.5% aqueous methyl cellulose at a dose volume of 10 ml/kg bw. Based on the results of this study, in the main micronucleus test groups of five male and five female mice were given a single oral gavage dose of either 0, 12.5, 25 or 50 mg/kg bw of Fipronil. The controls (0 mg/kg bw) received the vehicle alone. A similar sized positive control group was given 30 mg/kg bw of Chlorambucil in aqueous ethanol (10%). Dose preparations were analysed for the achieved concentration of the test material. Animals were observed daily for clinical signs of toxicity. Individual body weights were recorded prior to dosing and at termination. Five males and five females from each group were killed 24 hours after treatment. Additional groups of five males and five females, from the vehicle control and 50 mg/kg of Fipronil groups were killed 48 and 72 hours after treatment. Bone marrow smears were made from each animal, stained and examined microscopically. A total of at least 2000 erythrocytes per animal were examined for the presence of micronuclei, using the light microscope. The frequencies of micronucleated cells per 1000 polychromatic erythrocytes were analysed statistically. The ratio of polychromatic mature cells was also calculated for each animal.	X
5.2	**Results and discussion**	<u>Dose preparation analysis</u> In the preliminary toxicity test, achieved concentrations of Fipronil were between 89 and 100% of nominal. The mean achieved concentration at the highest dose level in the main micronucleus test was an acceptable 96% of nominal. Although results at the two lower dose levels of 25 and 12.5 mg/kg bw were 64 and 56% of nominal, they did not affect the scientific validity or integrity of the study.	

Section A6.6.4 **Annex Point IIA,** **VI.6.VI.6.4**	***In vivo* genotoxicity study (2nd micronucleus test)**	
	Preliminary toxicity test In the preliminary toxicity test, both males dosed with 120 mg/kg bw were found dead within about 18 hours of dosing. One of the males was hunched and underactive two hours after dosing. One female was hunched and underactive 2 hours post-dosing and both were hyperactive with unstable gait at about 18 hours. At 70 mg/kg bw, two mice (one of each sex) died. One male was found dead about 23 hours post-dosing having exhibited hunched posture at 18 hours. The other male exhibited transient piloerection, underactivity, hunched posture and convulsions at 18 hours but survived to scheduled termination. One female was found dead at about 42 hours post-dosing after showing hunched posture and underactivity on the day after treatment. The other female was unaffected. All mice, except for one female dosed at 30 mg/kg bw, lost weight after treatment with Fipronil. In the absence of any evidence of bone marrow toxicity, 50 mg/kg bw of Fipronil was selected as the highest dose level for the main micronucleus test. Micronucleus test (see Table A6.6.4.2-1) In the main micronucleus test, eight of the thirty mice treated with 50 mg/kg bw of Fipronil showed clinical signs of toxicity including hunched posture (8 mice), piloerection (5), convulsions (4), underactivity (3) and slow respiration (1). No clinical signs were seen at 12.5 and 25 mg/kg bw. One female given Chlorambucil as the positive showed hunched posture and underactivity 3 hours post-dosing. Most of the Fipronil high dose level mice lost weight after dosing. Body weight was unaffected at 12.5 and 25 mg/kg bw. The frequencies of micronucleated polychromatic erythrocytes in animals killed 24, 48 or 72 hours after treatment with Fipronil were similar to those in concurrent controls. Furthermore, the ratio of polychromatic to normochromatic (mature) erythrocytes was similar to control values. Therefore there was no evidence of bone marrow toxicity. The positive control compound, Chlorambucil, produced a significantly higher frequency of micronucleated polychromatic cells and a reduction in the ratio of polychromatic to mature erythrocytes thereby demonstrating the sensitivity of the assay.	
5.3 Conclusion	Fipronil showed no potential to induce chromosomal damage (clastogenicity as evidenced by micronuclei formation) and no evidence of bone marrow toxicity in mice following acute oral administration of up to 50 mg/kg bw.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A6.6.4.2-1 Summary micronucleus test evaluation

Exposure period	Treatment (mg/kg bw)		Sex	Micronucleated polychromatic cells / 1000		P : M
				Mean ± SD	Range	
24 h	Fipronil	0	M+F	1.2 ± 0.6	0.0 – 2.0	0.9
		12.5	M+F	0.8 ± 0.9	0.0 – 2.9	0.9
		25	M+F	1.1 ± 0.5	0.0 – 1.9	0.9
		50	M+F	0.6 ± 0.7	0.0 – 2.0	1.0
	Chlorambucil	30	M+F	48.2 ± 20.3**	28.0 – 87.5	0.6
48 h	Fipronil	0	M+F	0.6 ± 0.7	0.0 – 1.9	1.0
		50	M+F	0.9 ± 1.1	0.0 – 3.0	0.9
72 h	Fipronil	0	M+F	0.7 ± 0.8	0.0 – 2.0	1.0
		50	M+F	1.0 ± 1.1	0.0 – 3.1	0.7

P:M = Ratio of polychromatic : mature cells
Statistical evaluation: ** = p< 0.01 (Mann-Whitney U-test, one-tailed)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section 2*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *stable* The stability was the responsibility of the sponsor 3.2.5 Age/weight at study initiation: ~~*males 23.5 - 27.3 g females 18.8 - 23.1 g*~~ Males: 23.5-29.1 g; Females: 18.8-23.7 g 3.3.6 Vehicle: ~~*0.5% aqueous solution of carboxymethyl cellulose*~~ 0.5% aqueous methyl cellulose 3.3.7 Concentration in vehicle: *Main test:* ~~*0.125*~~ 1.25, *2.5, or 5 mg/ml* 3.5 Further remarks/5.1 Materials and methods: ~~*Body weights were determined prior to treatment and again immediately before termination*~~ All animals were weighed on the day of treatment and again immediately before termination. In addition, the animals in the preliminary toxicity test were weighed immediately prior to dosing and daily thereafter until termination.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *The ratio of polychromatic* cells to *mature* normochromatic *cells was also calculated for each animal.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.6.5 ***In vivo*** **mutagenicity test for DNA damage**
Annex Point IIA, VI.6.VI.6.5

		Official use only
	1. REFERENCE	
1.1 Reference	**A6.6.5/01** XXXX. In vivo unscheduled DNA synthesis (UDS) assay with BAS 350 I (Fipronil) in rat hepatocytes – single oral administration. XXXX (unpublished) (XXXX) **A6.6.5/02** 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. (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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes OECD No 486 EC 2000/32, B.39	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material		X
3.1.1 Lot/Batch number	COD 000082	
3.1.2 Specification	As given in Section 2	X
3.1.2.1 Description	White powder	
3.1.2.2 Purity	97.3%	
3.1.2.3 Stability	Stable	X
3.1.2.4 Maximum tolerable dose	50 mg/kg bw	
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Wistar	
3.2.3 Source	XXXX	
3.2.4 Sex	Male	
3.2.5 Age/weight at study initiation	10 -12 weeks mean bodyweight: 241 g	
3.2.6 Number of animals per group	3	
3.2.7 Controls animals	yes	
3.3 Administration/ Exposure		
3.3.1 Number of applications	1	
3.3.2 Interval between applications	Not applicable	

Section A6.6.5	***In vivo* mutagenicity test for DNA damage**	
Annex Point IIA, VI.6.VI.6.5		

3.3.3 Postexposure period	3 and 14 hours Oral	
3.3.4 Type	Gavage	
3.3.5 Concentration	3-h post-exposure group: 0, 25 and 50 mg/kg bw 14-h post-exposure group: 0, 12.5 and 25 mg/kg bw	
3.3.6 Vehicle	Corn oil	
3.3.7 Concentration in vehicle	0.125, 0.25, and 0.5 g/100 ml	
3.3.8 Total volume applied	10 ml/kg bw	
3.3.9 Controls	Yes Negative (vehicle) control and positive control	
3.3.14 Substance used as Positive Control	2-Acetylaminofluorene (AAF)	
3.4 Examinations		
3.4.1 Clinical signs	Yes	
3.4.2 Tissue	Primary hepatocytes isolated and cultured 3 or 14 hours after treatment of rats with Fipronil - Cytotoxicity (viability: trypan blue exclusion; cell morphology) - Quantification of UDS in hepatocytes after exposure to radiolabelled thymidine	X
3.5 Further remarks	–	
	4. RESULTS AND DISCUSSION	
4.1 Clinical signs	Range-finding experiment: In a pretest for the determination of the acute oral toxicity in males and females, deaths were observed down to a dose of 60 mg/kg bw. At 50 mg/kg bw, all animals survived showing no clinical signs. However, in the main test, animals treated with 50 mg/kg bw died unexpectedly before intended termination 14-h after treatment. Main test: Therefore 25 and 50 mg/kg bw were finally tested for the 3-h interval and 12.5 and 25 mg/kg bw for the 14-h interval. In these groups no symptoms or clinical signs were noted.	X
4.2 Haematology / Tissue examination	Primary hepatocytes: No treatment-related effect on cell viability or cell morphology of hepatocytes	
4.3 Genotoxicity	No	
4.4 Other	-	

Section A6.6.5	***In vivo* mutagenicity test for DNA damage**
Annex Point IIA, VI.6.VI.6.5	

5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was tested for its ability to induce DNA repair synthesis (unscheduled DNA synthesis; UDS) in vivo in rat hepatocytes. In the main test, groups of 3 male rats were administered the vehicle, 12.5. 25 or 50 mg/kg bw Fipronil, or the positive control substance 50 mg/kg 2-AAF by oral gavage in a volume of 10 ml/kg bw. The animals were surveyed for evident clinical signs of toxicity throughout the study. Three or 14 hours after treatment, rats were anesthetized, the livers removed and hepatocytes isolated. About 400,000 viable hepatocytes (as determined by trypan blue exclusion) were seeded on coverslips. After an attachment period of about 2 hours, cells were exposed for approx. 4 h to labeling medium containing radiolabelled thymidine, followed by a 14-h incubation in medium containing unlabelled thymidine. Subsequently, cells were fixed, coverslips were mounted and slides were processed for autoradiography. <u>Quantification of UDS:</u> After coding three slides per test group were examined microscopically. In order to achieve a total number of 100 cells/animal, 25-50 cells of good morphological condition were randomly selected from each slide. By means of an automatic image analyzer the nuclear grain count (NG) and the cytoplasmic grain count (CG) was determined. The cytoplasmic area was adjacent to the nucleus and had approximately the 2 to 3 fold size of the nucleus. The following parameters were calculated: the net nuclear grain count of each cell (NNG = NG – CG) the mean nuclear grain count (NG) the mean cytoplasmic grain count (CG) the mean net nuclear grain count (mean NNG) the percentage of cells in repair (cells with NNG ≥ 0) the percentage of cells in repair (cells with NNG ≥ 5) Evaluation criteria: The test substance is considered positive in this test if a dose-related increase is demonstrated in both of the following: The mean number of NNG counts, which must exceed zero at one of the test points. The percentage of cells in repair (NNG ≥ 5) when ≥ 20. A dose-related increase in % cells in repair ≥ 5 outside the values of both the concurrent negative control and the historical control data base (≥ 5 < 20) and a dose-related increase in the mean number of NNG counts near to but without exceeding zero is considered to be an indication for a marginal response which needs to be confirmed / clarified in a further experiment. A test article producing both NNG counts and % cells in repair in the range of the negative control data is considered to be negative in the in vitro UDS assay.	

Section A6.6.5	***In vivo* mutagenicity test for DNA damage**
Annex Point IIA, VI.6.VI.6.5	

5.2	**Results and discussion**	Analytical results The stability of a comparable batch (78/GC/90) in the vehicle corn oil has been verified analytically. The homogeneity of the test substance in the vehicle was guaranteed by constant stirring during the removal and administration of the test substance formulation and by analytical determination of three individual samples of each concentration. Depending on the dose, the analytically determined concentrations were 105-108% of the theoretical values. Clinical findings Clinical examination of the rats after administration of the test- and control-substances revealed no signs of systemic toxicity, with the exception of one male rat administered 25 mg/kg bw Fipronil, which showed tonic-clonic convulsions about three hours after Fipronil administration. Cytotoxicity Cell viability was not influenced by treatment (94.4–102.6% of control values). Morphological changes of the cells were not observed. UDS assay (see Table A6.6.5-1) In hepatocytes harvested either 3 hours or 14 hours after administration of Fipronil, no increase in the number of net nuclear grain counts or in the percentage of cells in repair was noted at any dose. In contrast, 2-AAF treatment led to a marked increase in the number of net nuclear grains as well as the percentage of cells in repair, thus demonstrating the sensitivity of the test system.	X
5.3	**Conclusion**	Under the experimental conditions chosen, the results indicate that Fipronil did not cause any increase in unscheduled DNA synthesis, as measured by an increase in net nuclear grain counts, and it was concluded that the test substance was negative in this in vivo of the UDS assay using rat hepatocytes.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

X

~~Table A6.6.5-1~~ Table A6.6.5.1-1 Results of UDS assay in rat hepatocytes

Test groups	Fipronil dose [mg/kg bw]				Pos. control
	0	12.5	25	50	50 mg/kg bw 2-AAF
Sacrifice 3 hours after treatment					
NG counts Mean ± SD*	3.66 ± 0.59	–	3.66 ± 0.64	2.99 ± 0.45	11.75 ± 5.06
CG counts Mean ± SD*	9.28 ± 1.27	–	8.03 ± 1.33	7.21 ± 0.79	5.52 ± 3.16
NNG counts Mean ± SD*	-5.62 ± 1.06	–	-4.37 ± 0.96	-4.22 ± 0.73	6.24 ± 1.91
% cells in repair – NNG ≥ 0 Mean ± SD*	4.67 ± 3.06	–	6.00 ± 6.00	7.00 ± 2:65	99.33 ± 0.58
% cells in repair NNG ≥ 5 Mean ± SD*	0.67 ± 0.58	–	0.00 ± 0.00	0.00 ± 0.00	54.67 ± 11.24
Sacrifice 14 hours after treatment					
NG counts Mean ± SD*	3.56 ± 0.70	3.01 ± 0.45	3.66 ± 0.91	–	13.89 ± 2.31
CG counts Mean ± SD*	8.09 ± 1.02	8.21 ± 0.79	8.86 ± 1.28	–	7.35 ± 1.35
NNG counts Mean ± SD*	-4.54 ± 0.47	-5.20 ± 0.51	-5.20 ± 0.77	–	6.53 ± 1.20
% cells in repair – NNG ≥ 0 Mean ± SD*	7.00 ± 1.73	3.67 ± 2.52	3.33 ± 2.08	–	94.33 ± 3.21
% cells in repair NNG ≥ 5 Mean ± SD*	0.33 ± 0.58	0.00 ± 0.00	0.00 ± 0.00	–	65.33 ± 13.20

NG = nuclear grains
CG = cytoplasmic grains
NNG = net nuclear grains
* = mean and standard deviation per group
(mean of three animals; each animal datum representing the mean of findings in 100 cells)

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: Fipronil : BAS 350 I 3.1.2 Specification: ~~*As given in section 2*~~ none 3.1.2.3 Stability: *Stable* The stability of the test substance was determined by reanalysis. 3.4.2 Tissue: *Primary hepatocytes isolated and cultured 3 or 14 hours after treatment of rats with Fipronil* 3 and 14 hours after treatment of rats with Fipronil, the livers were removed and the hepatocytes were isolated:
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Clinical signs: *However, in the main test, animals treated with 50 mg/kg bw died unexpectedly before intended termination 14-h after treatment* (the day after test substance administration).
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *UDS assay* ~~*(see Table A6.6.5-1)*~~ (see Table A6.6.5.1-1)
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in rats**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.7/01** 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. XXXX (unpublished) (XXXX)	
Remark:		This two-year combined chronic toxicity / carcinogenicity study in rats is summarised in Section A6.5 (see Reference A6.5/01)	

	EVALUATION BY COMPETENT AUTHORITIES
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	See the two-year combined chronic toxicity / carcinogenicity study in rats is summarised in Section A6.5 (see Reference A6.5/01)
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.7	**Carcinogenicity study**
Annex Point IIA, VI.6.7	**– in mice**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A6.7/02** XXXX. M&B 46030: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. Final Report. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EU 88/302/EEC, B.32 (1988) USEPA (=EPA) 83-5 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	An interim kill to assess chronic toxicity is not standard	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	CD-1	
3.2.3	Source	XXXX	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	34 to 41 days of age Males 24 – 30 g Females 19 – 28 g	**X**
3.2.6	Number of animals per group		
3.2.6.1	at interim sacrifice	20 animals/group/sex	
3.2.6.2	at terminal sacrifice	52 animals/group/sex	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	**Oral**	
3.3.1	Duration of treatment	78 weeks	
3.3.2	Interim sacrifice(s)	53 weeks	
3.3.3	Final sacrifice	78 weeks	
3.3.4	Frequency of exposure	Continuous	
3.3.5	Post exposure period	None	
3.3.6	Type	Oral In diet	

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	
3.3.7 Concentration	0, 0.1, 0.5, 10, 30 ppm, corresponding to intakes Males: 0, 0.011, 0.055, 1.181, 3.43 mg/kg bw/d Females: 0, 0.012, 0.063, 1.230, 3.616 mg/kg bw/d A further test group at 60 ppm was terminated due to mortality in week 10	
3.3.8 Vehicle	None	
3.3.9 Concentration in vehicle	Not applicable	
3.3.10 Controls	Plain diet	
3.4 Examinations		
3.4.1 Body weight	Yes / weekly for 14 weeks, fortnightly thereafter and at necropsy	x
3.4.2 Food consumption	Yes / weekly	
3.4.3 Water consumption	Observed daily – no quantative measurements made	
3.4.4 Clinical signs	Yes / twice daily	x
3.4.5 Macroscopic investigations	Yes / weekly and at necropsy	
3.4.6 Ophthalmoscopic examination	No	
3.4.7 Haematology	Yes	
	Number of animals: All animals from oncogenicity phase (52 animals/group/sex)	x
	Time points: Week 50 and 76	
	Parameters: Differential leukocyte count	x
3.4.8 Clinical chemistry	No	
3.4.9 Urinalysis	No	
3.4.10 Pathology	yes	
3.4.10.1 Organ weights	Yes	
	from: All animals	
	Organs: Adrenals, brain, heart, kidney, liver, lungs (with mainstem bronchi), spleen, testes, uterus with cervix	
3.4.11 Histopathology		
	at interim sacrifice (53 weeks)	
	from: all dose groups	
	Organs: Kidneys, liver, lungs with mainstem bronchi	
	from high dose group and controls	
	Organs: thyroid with parathyroid	
	at terminal sacrifice (78 weeks)	
	from: all dose groups	
	Organs: Kidneys, liver, lungs with mainstem bronchi, tissues reported at macrocscopic examination as being abnormal	
	from high dose group and controls	

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**		
	Organs:	Adrenals; aorta – thoracic; brain; caecum; colon; duodenum; epididymides; eye and optic nerve, left; femoral bone and marrow; gall bladder; heart; ileum; jejunum; lymph nodes, – mandibular and – mesenteric; mammary gland – caudal; oesaphagus; ovaries; pancreas; pituitary; prostate; rectum; salivary gland; – left submandibular; sciatic nerve, left; seminal vesicles; skeletal muscle – thigh; skin; spinal cord; spleen; sternum; stomach – keratinised and – glandular; testes; thymus; thyroid with parathyroids; trachea; urinary bladder; uterus with cervix; vagina.	
	From Organs:	**All mice not surviving to scheduled sacrifice** Adrenals; aorta – thoracic; brain; caecum; colon; duodenum; epididymides; eye and optic nerve, left; femoral bone and marrow; gall bladder; heart; ileum; jejunum; kidneys; liver; lungs with mainstem bronchi; lymph nodes, – mandibular and – mesenteric; mammary gland – caudal; oesaphagus; ovaries; pancreas; pituitary; prostate; rectum; salivary gland; – left submandibular; sciatic nerve, left; seminal vesicles; skeletal muscle – thigh; skin; spinal cord; spleen; sternum; stomach – keratinised and – glandular; testes; thymus; thyroid with parathyroids; trachea; urinary bladder; uterus with cervix; vagina.; tissues reported at macrocscopic examination as being abnormal	
3.4.12 Other examination	Palpable swellings		x
3.5. Statistics	Intergroup differences in mortality: Cox proportional hazard model, Tarone's partition of Chi-Square statistic into linear trend on dose and deviation from linearity (2-tailed) Differential blood cell count: Mann-Whitney Test (2-tailed) Organ and body weight changes: Bartlett's test + Behrens-Fisher (if homogeneous otherwise Dunnett's-Test Non-neoplastic macroscopy and histopathology findings: Fisher's Exact Test (2-tailed) Neoplastic macroscopy and histopathology findings: Fisher's Exact Test (1-tailed) Statistically not significant: $p > 0.05$		
3.6 Further remarks	None		
4.1 Body weight	**4. RESULTS AND DISCUSSION** (See Table A6.7-1) At 60 ppm bodyweight gains were reduced compared with controls during the first 9 weeks of treatment. At 30 ppm, low weight gains were also recorded and overall values were 14% (males) and 19% (females) below controls. At 10 ppm, gains of males were low during the first 13 weeks whilst females were affected during the first 26 weeks. Bodyweights at 0.1 and 0.5 ppm were unaffected.		x

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	

4.2	**Food consumption**	(See Table A6.7-2) At 60 ppm, food consumption in males was reduced during the first 2 weeks of treatment only, but, intake in females was low during the first 9 weeks. At 30 ppm, food consumption in both sexes was slightly, but consistently lower than controls. No clear effect was seen at 10 ppm or below. As expected, at 60 ppm, food conversion was lower than controls for the first 9 weeks of treatment. Overall efficiency of conversion was low for the first 14 weeks in males at 10 and 30 ppm and for the first two weeks in females alone at 30 ppm.	**X**
4.3	**Water consumption**	Not recorded	
4.4	**Clinical signs**	<u>Mortality</u> During the first 9 weeks of treatment, 14 males and 7 females given 60 ppm died. Although one male had a convulsion before death, no significant ante mortem signs were seen in the other animals. Necropsy did not reveal any contributory lesions. It was concluded that their deaths were due to treatment with Fipronil. Consequently, all surviving animals from this group were killed without necropsy during Week 10. No treatment-related increase in mortality was seen in the other dose groups. Clinical signs Clinical signs were confined to convulsive episodes during Week 2 in three males fed 60 ppm, which resulted in death of one animal. There were no other treatment-related signs.	
4.5	**Macroscopic investigations**	Considering decedents and those killed after 78 weeks of treatment together, the incidences of liver enlargement and of changes on the surface of the liver were higher in males given 30 ppm than in controls. Additionally at this dose level, incidences of prominent splenic white pulp and obesity in females and hairloss in males were lower. In decedents fed 30 ppm, the incidences of oedema of the subcutis and perineal staining win females were lower. No treatment related macroscopic changes were seen in the Toxicity phase animals.	
4.6	**Ophthalmoscopic examination**	Not recorded	
4.7	**Haematology**	Examination of the blood smears obtained after 50 and 78 weeks of treatment showed no clear treatment related haematological changes. The slightly lower proportion of neutrophils and slightly higher proportion of lymphocytes after 76 weeks seen in females given 30 ppm was not apparent after 50 weeks and was not seen in males. Therefore they could not be conclusively related to treatment.	
4.8	**Clinical chemistry**	Not recorded	
4.9	**Urinalysis**	Not recorded	

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	
4.10 Pathology	(See Table A6.7-5) Considering decedents and those killed after 78 weeks of treatment together, the incidences of liver enlargement and of changes on the surface of the liver were higher in males given 30 ppm than in controls. Additionally at this dose level, incidences of prominent splenic white pulp and obesity in females and hair-loss in males were lower. In decedents fed 30 ppm, the incidences of oedema of the subcutis and perineal staining in females were lower. No treatment-related macroscopic changes were seen in the toxicity phase animals.	**X**
4.11 Organ weights	(See Table A6.7-4) After 53 and 78 weeks of treatment increased bodyweight-relative liver weights compared with controls occurred in males given 10 ppm and in both sexes fed 30 ppm. In males, absolute liver weights were increased on both occasions at 10 and 30 ppm and after 53 weeks in females given 30 ppm.	**X**
4.12 Histopathology	(See Table A6.7-6) <u>Histopathology, 53-week satellite animals ("Toxicity phase")</u> At 10 and 30 ppm, the incidence of periacinar microvesicular vacuolation in the liver of males killed after 53 weeks of treatment was significantly increased. <u>Histopathology, 78-week animals ("Oncogenicity phase")</u> At 30 ppm, the incidences of hepatocellular hyperplasia and chronic degenerative changes in the liver of decedent males were increased. Degenerative changes included necrosis of occasional cells and apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes, chronic inflammation and bile stasis. All other findings were considered to be fortuitous. <u>Animals killed after 78 weeks of treatment</u> At 10 and 30 ppm, the incidence of periacinar microvesicular vacuolation of hepatocytes in males was significantly higher than in controls. This was also seen in females at 0.5 ppm and above although females given 0.5 or 10 ppm had a lower incidence of periacinar fatty vacuolation.	**X**
4.13 Other examinations		
4.14 Time to tumours	No increase in tumours over controls	
4.15 Other		

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was administered via the diet to CD-1 mice for up to 78 weeks to assess its oncogenic potential. A satellite group was also included to evaluate its chronic toxicity after up to 53 week treatment. Dietary concentrations were selected on the basis of a preliminary study in which dietary concentrations of 110 ppm and above were associated with high mortality, overactivity/irritability and convulsions, low food intake, poor weight performance, inferior food efficiency and high liver weights. At 40 ppm 2/24 animals died and at 15 and 40 ppm there was inferior food intake, weight gain, food conversion efficiency and high liver weights. At the start of treatment, the animals were 34 to 41 days of age and weighed 24 to 30 g (males) and 19 to 28 g (females). They were acclimatised for 13 days before the start of treatment and were housed by sex in groups of 4. Batches of test diets were prepared at weekly intervals by directly mixing the test material with the diet. Prior to the start of treatment, homogeneity of mixing Fipronil with the diet and its stability in this medium at 0.1 ppm were assessed in a trial mix. Stability at 60 ppm had been demonstrated in a previous study. In addition, samples from the present study were taken during Weeks 1-4 and at eight-week intervals for analysis of the achieved test material concentration. For the oncogenicity part of the study, groups of 52 male and 52 female CD-1 mice were given dietary concentrations of 0, 0.1, 0.5, 10, 30 or 60 ppm of Fipronil for at least 78 weeks. A further 20 male and 20 female mice per group were treated for 53 weeks then killed to assess chronic toxicity (toxicity phase). In view of the high mortality at 60 ppm, all surviving animals in this group were killed, without necropsy, in Week 10. Animals were observed at least twice daily for mortality and clinical signs. A detailed weekly examination, including a palpation, was also performed. Individual bodyweights were recorded weekly for the first 14 weeks of treatment, fortnightly thereafter and at necropsy. Food consumption for each cage group was recorded weekly, together with an estimate of spillage. Blood smears for hematology were taken from all oncogenicity phase animals after 50 and 76 weeks of treatment. All animals were necropsied, examined macroscopically externally and internally (cranial, thoracic, abdominal and pelvic cavities and their contents). Selected organs were weighed and a comprehensive range of tissues preserved. All tissues (except the right eye and optic nerve, Harderian glands, cranial mammary gland, right submandibular salivary gland, right sciatic nerve and tongue) from the control and highest dose groups of the oncogenicity phase and all decedents were examined microscopically. The liver, lungs and kidneys from the other dose groups from this phase and from the toxicity phase animals as well as abnormalities from all animals were examined, too.	**x**

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	
5.2 Results and discussion	Analysis of diet preparations Acceptable homogeneity and stability of Fipronil were confirmed. The concentrations of Fipronil in diet administered in Weeks 1-4 and at eight-week intervals thereafter averaged 100+11%, 92+11%, 88+7% and 90+6% of nominal at 0.1, 0.5, 10 and 30 ppm, respectively. Achieved concentrations was generally within + 15% of nominal. The few deviations from this were considered not to have affected the integrity of the study. Mortality During the first 9 weeks of treatment, 14 males and 7 females given 60 ppm died. Although one male had a convulsion before death, no significant *ante mortem* signs were seen in the other animals. Necropsy did not reveal any contributory lesions. It was concluded that their deaths were due to treatment with Fipronil. Consequently, all surviving animals from this group were killed without necropsy during Week 10. No treatment-related increase in mortality was seen in the other dose groups. Clinical signs Clinical signs were confined to convulsive episodes during Week 2 in three males fed 60 ppm and which resulted in death in one animal. There were no other treatment-related signs. Palpable masses There was no treatment-related effect upon the location, multiplicity or mean time of onset of palpable swellings. Bodyweight (See Table A6.7-1) At 60 ppm, bodyweight gains were reduced compared with controls during the first 9 weeks of treatment. At 30 ppm, low weight gains were also recorded and overall values were 14% (males) and 19% (females) below controls. At 10 ppm, gains of males were low during the first 13 weeks whilst females were affected during the first 26 weeks. Bodyweights at 0.1 or 0.5 ppm were unaffected. Food consumption (See Table A6.7-2) At 60 ppm, food consumption in males was reduced during the first 2 weeks of treatment only, while food intake in females was low during the first 9 weeks. At 30 ppm, food consumption in both sexes was slightly, but consistently lower than controls. No clear effect was seen at 10 ppm or below. As expected, at 60 ppm, food conversion was lower than controls for the first 9 weeks of treatment. Overall efficiency of conversion was low for the first 14 weeks in males at 10 and 30 ppm and for the first two weeks in females alone at 30 ppm. Test substance intake (See Table A6.7-3) Hematology Examination of the blood smears obtained after 50 and 76 weeks of treatment showed no clear treatment-related hematological changes. The slightly lower proportion of neutrophils and slightly higher proportion of lymphocytes after 76 weeks seen in females given 30 ppm was not apparent after 50 weeks and was not seen in males. Therefore they could not be conclusively related to treatment.	x x x

Section A6.7 **Annex Point IIA, VI.6.7**	**Carcinogenicity study** **– in mice**	
	Organ weights (See Table A6.7-4) After 53 and 78 weeks of treatment, increased bodyweight-relative liver weights compared with controls occurred in males given 10 ppm and in both sexes fed 30 ppm. In males, absolute liver weights were increased on both occasions at 10 and 30 ppm and after 53 weeks in females given 30 ppm.	**X**
	Macropathology (See Table A6.7-5) Considering decedents and those killed after 78 weeks of treatment together, the incidences of liver enlargement and of changes on the surface of the liver were higher in males given 30 ppm than in controls. Additionally at this dose level, incidences of prominent splenic white pulp and obesity in females and hair-loss in males were lower. In decedents fed 30 ppm, the incidences of oedema of the subcutis and perineal staining in females were lower. No treatment-related macroscopic changes were seen in the toxicity phase animals.	**X**
	(See Table A6.7-6) Histopathology, 53-week satellite animals ("Toxicity phase") At 10 and 30 ppm, the incidence of periacinar microvesicular vacuolation in the liver of males killed after 53 weeks of treatment was significantly increased. Histopathology: animals killed after 78 weeks of treatment At 10 and 30 ppm, the incidence of periacinar microvesicular vacuolation of hepatocytes in males was significantly higher than in controls. This was also seen in females at 0.5 ppm and above although females given 0.5 or 10 ppm had a lower incidence of periacinar fatty vacuolation. Histopathology, 78-week decedent animals ("Oncogenicity phase") At 30 ppm, the incidences of hepatocellular hyperplasia and chronic degenerative changes in the liver of decedent males were increased. Degenerative changes included necrosis of occasional cells and apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes, chronic inflammation and bile stasis. All other findings were considered to be fortuitous.	**X**
5.3 Conclusion	Dietary treatment with 60 ppm of Fipronil caused convulsive episodes resulting in death of several mice, marked reductions in bodyweight and food intake. Therefore this dose level was terminated during Week 10. Reductions occurred in bodyweight gain and food consumption as well as increased liver weights, associated with liver enlargement and surface changes, hepatic hyperplasia, chronic hepatic degenerative changes including microvesicular periacinar vacuolation. At 10 ppm, slightly low bodyweight gain over the first 26 weeks, increased liver weights in males and an increased incidence of microvesicular vacuolation were seen. Dietary administration of up to 30 ppm of Fipronil to mice for 78 weeks showed no oncogenic potential. The NOEL was 0.5 ppm, corresponding to mean intakes of 0.055 and 0.063 mg/kg bw/d of Fipronil in males and females, respectively.	**X**
5.3.1 Reliability	1	

Section A6.7	**Carcinogenicity study**
Annex Point IIA, VI.6.7	**– in mice**

5.3.2 Deficiencies	None	

~~**Table A6.7-1**~~ **Table A6.7.2-1** **Group mean bodyweight change (g)** X

Weeks	Dose level (ppm)									
	0	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
	Males					**Females**				
0-13	15.9 (100)	14.7 (92)	15.1 (95)	14.0* (88)	11.7** (74)	10.9 (100)	10.5 (96)	10.7 (98)	9.1* (83)	9.1* (83)
13-26	4.9 (100)	5.0 (102)	5.3 (108)	5.9 (120)	4.4 (90)	6.7 (100)	6.4 (96)	7.4 (110)	5.8 (87)	5.9 (88)
0-26	21.1 (100)	19.8 (94)	20.5 (97)	20.0 (95)	16.1** (76)	17.6 (100)	16.9 (96)	18.1 (103)	14.9 (85)	14.7* (84)
0-52	23.4 (100)	23.0 (98)	23.0 (98)	22.0 (94)	19.7** (84)	22.2 (100)	21.0 (95)	23.7 (107)	21.1 (95)	19.1 (86)
0-78	23.1 (100)	21.9 (95)	23.7 (103)	21.3 (92)	19.9 (86)	25.4 (100)	21.7 (85)	25.8 (102)	22.1 (87)	20.6* (81)

% of control in parenthesis
Statistical evaluation:* $p \leq 0.05$ ** $p \leq 0.01$

~~**Table A6.7-2**~~ **Table A6.7.2-2** **Group mean food consumption (g/animal/week)** X

Weeks	Dose level (ppm)									
	0	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
	Males					**Females**				
Weekly consumption										
1	32	30	31	30	30	31	29	28	29	29
2	34	33	34	32	32	35	32	31	33	31
4	33	32	31	29	30	34	31	32	30	29
8	38	37	37	35	35	37	36	37	36	34
Total food consumed										
1-13	465 (100)	453 (97)	450 (97)	444 (95)	433 (93)	472 (100)	447 (95)	456 (97)	442 (94)	422 (89)
1-26	950 (100)	928 (98)	918 (97)	908 (96)	878 (92)	958 (100)	903 (94)	932 (97)	886 (92)	841 (88)
1-52	1951 (100)	1885 (97)	1879 (96)	1892 (97)	1804 (92)	1864 (100)	1726 (93)	1813 (97)	1712 ~~(99)~~ (92)	1621 (87)
1-78	2971 (100)	2848 (96)	2870 (97)	2915 (98)	2753 (93)	2774 (100)	2556 (92)	2722 (98)	2517 (91)	2391 (86)

X

% of control in parenthesis

~~Table A6.7-3~~ Table A6.7.2-3 Group test material intake (mg/kg/day) X

Weeks	Dose level (ppm)									
	Males					Females				
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
1 – 78	0	0.011	~~0.053~~ 0.055	1.181	3.430	0	0.012	0.063	1.230	3.616

X

~~Table A6.7-4~~ Table A6.7.2-4 Group mean liver weights (g) X

Weight	Dose level (ppm)									
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
	Males					Females				
Week 54: Absolute liver weight (g)	2.61 (100)	2.64 (101)	2.70 (103)	3.02 (116)	3.37** (129)	1.67 (100)	1.77 (106)	1.86 (111)	1.84* (110)	2.00** (120)
Week 54: Relative liver weight (%)	5.079 (100)	5.663 (111)	5.719* (113)	6.132* (121)	7.675** (151)	4.363 (100)	4.417 (101)	4.497 (103)	4.666 (107)	5.392** (124)
Week 79: Absolute liver weight (g)	2.77 (100)	2.78 (100)	2.92 (105)	3.30 (119)	3.81** (138)	1.99 (100)	1.93 (97)	2.06 (104)	2.02 (102)	2.13 (102)
Week 79: Relative liver weight (%)	5.634 (100)	5.744 (102)	5.977 (106)	7.008* (124)	8.261** (147)	4.5356 (100)	4.575 (101)	4.451 (98)	4.799 (106)	5.294** (117)

% of control in parenthesis
Statistical evaluation: * $p<0.05$ ** $p<0.01$

~~Table A6.7-5~~ Table A6.7.2-5 Group Incidence of macroscopic findings – Oncogenicity phase X

Finding	Dose level (ppm)									
	0	0.1	0.5	10	30	0	0.1	0.5	10	30
	Males					Females				
Liver:										
- Areas of change	0/52	1/52	3/52	3/52	8/52**	0/52	0/52	1/52	0/52	1/52
- Appears large	2/52	2/52	1/52	4/52	7/52	1/52	0/52	1/52	0/52	1/52
Spleen:										
- Prominent white pulp	5/52	6/52	4/52	3/52	7/52	7/52	11/52	6/52	3/52	0/52*
Obesity	~~0/52~~ 1/52	1/52	~~1/52~~ 0/52	~~0/52~~ 1/52	~~0/52~~ 2/52	~~5/52~~ 10/52	~~3/52~~ 4/52	~~6/52~~ 7/52	~~3/52~~ 5/52	~~1/52~~ 2/52*
Hairloss (moderate)	34/52	26/52	29/52	27/52	23/52*	12/52	11/52	14/52	11/52	10/52

X

Statistical evaluation: * $p\leq0.05$ ** $p\leq0.01$

~~Table A6.7-6~~ Table A6.7.2-6 **Group incidence of hepatic findings**

X

Finding	Dose level (ppm)									
	0	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
	Males					**Females**				
Microvesicular periacinar vacuolation										
After 53 weeks	0/14	2/15	2/19	7/16**	12/18***	1/18	1/19	4/15	1/17	4/13
After 78 weeks	5/24	7/31	7/26	13/26*	13/26*	0/32	0/32	4/26*	3/37	7/38*
Decendents: Hepatocellular hyperplasia										
	0/28	2/21	1/26	0/26	4/26*	0/20	0/20	0/26	0/15	0/14
Decedents: Chronic degenerative change										
	3/28	5/21	3/26	5/26	11/26*	6/20	4/20	5/26	4/15	2/14

Statistical evaluation: * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.2.5 Age/weight at study initiation/5.1 Materials and methods: *Males* ~~*24 – 30 g*~~ 23-32 g 3.4.4 Clinical signs: *Yes / twice daily* In addition a more detailed weekly examination, which included palpation, was performed on each animal. 3.4.1 Body weight: *Yes /* on the day that treatment commenced, *weekly for 14 weeks, fortnightly thereafter and at necropsy* 3.4.7 Haematology: *Number of animals:* ~~*All animals from oncogenicity phase (52 animals/group/sex)*~~ All animals in groups 1 and 5. *Parameters: Differential leukocyte count* Examination of the blood film 3.4.12 Other examination: *Palpable swellings,* mortality
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Body weight/5.2 Results and discussion : ~~*(See Table A6.7-1)*~~ (See Table A6.7.2-1) 4.2 Food consumption/5.2 Results and discussion: ~~*(See Table A6.7-2)*~~ (See Table A6.7.2-2) 4.10 Pathology/5.2 Results and discussion: ~~*(See Table A6.7-5*~~) (See Table A6.7.2-5) Distension of the gall bladder was seen after 78 weeks in a number of females from each treated group, this change was not observed in any control females. However, the incidence of this finding was not dosage-related and it is considered to be of no toxicological significance. 4.11 Organ weights/5.2 Results and discussion: ~~*(See Table A6.7-4)*~~ (See Table A6.7.2-4) The bodyweight-relative liver weights of animals receiving 60 ppm which died prematurely were clearly higher than expected in mice of this age. Slightly high bodyweight-relative liver weights after 53 weeks in males receiving 0.5 ppm were, in the absence of any similar changes after 78 weeks, not clearly attributable to treatment. 4.12 Histopathology/5.2 Results and discussion: ~~*(See Table A6.7-6*~~) (See Table A6.7.2-6) Toxicity phase: The increase in the incidence of periacinar microvesicular vacuolation was seen in some females treated at 0.5 or 30 ppm but there was no clear dosage-relationship.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *Test substance intake* ~~*(See Table A6.7-3)*~~ (See Table A6.7.2-3)

	5.3 Conclusion: At 30 ppm, *Reductions occurred in bodyweight gain and food consumption as well as increased liver weights, associated with liver enlargement and surface changes, hepatic hyperplasia, chronic hepatic degenerative changes including microvesicular periacinar vacuolation. At 10 ppm, slightly low bodyweight gain over the first 26 weeks* in females and during the first 13 weeks in males, *increased liver weights* (absolute and relative) *in males* after 53 and 78 weeks *and an increased incidence of microvesicular vacuolation were seen* after 53 and 78 weeks in males and after 78 weeks in females. *The ~~NOEL~~*NOAEL *was 0.5 ppm, corresponding to mean intakes of 0.055 and 0.063 mg/kg bw/d of Fipronil in males and females, respectively.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8 **Annex Point IIA, VI.6.8**	**Reproductive toxicity**

Section A6.8.1 **Annex Point IIA, VI.6.8.1**	**Teratogenicity test** **– Rat**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.8.1/01** XXXX. The effect of M&B 46030 on pregnancy of the rat. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**		
2.2	**GLP**	Yes	
2.3	**Deviations**	None that compromised the validity of the study results	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	JJW 2070	**X**
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	White powder	
3.1.2.2	Purity	95.1% (see analysis data given on study report pages 124 ff)	
3.1.2.3	Stability	Stable	**X**
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague Dawley CD	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 8-10 weeks Weight: 170 and 228 g	
3.2.6	Number of animals per group	25	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Time-mated females were supplied by the breeder. Day of mating = Day 0 of pregnancy (judged by presence of vaginal plug or appearance of sperm in vaginal smear)	
3.3	**Administration/ Exposure**	**Oral**	
3.3.1	Duration of exposure	From Days 6 to 15 *post coitum* (*p.c.*)	
3.3.2	Post exposure period	Day 16 to day 20 *p.c.*	
3.3.3	Type	Oral gavage	
3.3.4	Concentration	0 (control), 1, 4 or 20 mg/kg bw/d	
3.3.5	Vehicle	0.5% aqueous methylcellulose	
3.3.6	Concentration in vehicle	0.1, 0.4, 2 mg/ml	**X**
3.3.7	Total volume applied	10 ml/kg bw	

Section A6.8.1 **Annex Point IIA, VI.6.8.1**	**Teratogenicity test** **– Rat**	
3.3.8 Controls	Vehicle	
3.4 Examinations		
3.4.1 Body weight	Yes Days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 *post coitum* (*pc*).	
3.4.2 Food consumption	Yes: Food consumption was measured from weigh day to weigh day. Water consumption was measured daily from day 2.	
3.4.3 Clinical signs	Yes Daily	
3.4.4 Examination of uterine content	Number of corpora lutea, number of implantations; pre- and post implantation loss	
3.4.5 Examination of foetuses		
3.4.5.1 General	Number and distribution of embryfetal deaths classified as early (only placenta visible at termination) or late (both placental and embryonic remnants visible at termination); number and distribution of live young; individual fetal weights; sex ratio; fetal abnormalities	
3.4.5.2 Skeletal	Yes	
3.4.5.3 Soft tissues	Yes	
3.5 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Maternal toxic effects	Clinical signs No signs were noted. Bodyweight (see Tables A6.8.1.1-1 and -2) At the highest dose level (20 mg/kg bw/d), bodyweight gain was reduced from the start of dosing (Day 6 pc) until Day 10 pc. Marginally lower bodyweight gain at 4 mg/kg bw/d from Day 6 pc throughout the study, was considered of doubtful biological significance. Food intake and water consumption (see Tables A6.8.1.1-3 and -4) At 20 mg/kg bw/d, food consumption was low during treatment days 6–11 *p.c.* but was unaffected thereafter; water consumption was slightly increased from Day 8 *p.c.* to termination. Abortion and total litter loss (see Table A6.8.1.1-5) There were no effects of maternal treatment upon litter size and survival at any dose level. Terminal necropsy findings There were no treatment-related macroscopic findings at any dose level.	x
4.2 Teratogenic / embryotoxic effects	(See Table A6.8.1.1-5) There were no adverse effects of treatment at any dosage on embryo-fetal losses, litter size, litter and mean fetal weights and sex ratio. There were no treatment-related differences in the incidence of malformation, skeletal or visceral anomalies when compared to controls. Intergroup differences in the incidence o£ skeletal variants did not reveal any significant effects of treatment.	
4.3 Other effects		
	5. APPLICANT'S SUMMARY AND CONCLUSION	

Section A6.8.1 **Annex Point IIA, VI.6.8.1**	**Teratogenicity test** **– Rat**

5.1	**Materials and methods**	Fipronil was administered by oral gavage to groups of pregnant rats according to the following study design in order to assess its potential to affect development of the conceptus *in utero*. Groups of 25 mated Sprague Dawley CD rats were given a daily oral dose, by gavage, of either 0 (control), 1, 4 or 20 mg/kg bw/d from Days 6 to 15 *post coitum* (*p.c.*) at a dose volume of 10 ml/kg bw/d. Control animals were given the vehicle alone, 0.5% methylcellulose. The date of mating (sperm in the vaginal smear) was designated Day 0 *p.c.* The time mated rats were 8-10 weeks old (Day 1 or 2 *p.c.*) on arrival and weighed between 170 and 228 g on Day 2 *p.c.* At the start of dosing (Day 6 *p.c.*) they weighed between 207 and 280 g. They were housed five to a cage. Animals were examined daily for mortality and clinical signs of toxicity. Individual bodyweights were recorded on Days 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 *p.c.* Food and water consumption were recorded throughout gestation between each weight determination day (food intake or daily water intake). The females were killed on Day 20 *p.c.* and examined macroscopically for abnormalities. The uterus of apparently not pregnant females was stained with ammonium sulfide to reveal possible implantation sites. For pregnant females, the numbers of corpora lute and implantations were recorded together with the type and uterine position of each implant. Live fetuses were weighed, sexed and examined for external abnormalities then preserved in either alcohol or Bouin's fluid. Half of the fetuses (those preserved in alcohol) were eviscerated and examined for skeletal abnormalities and the remainder were examined for visceral anomalies by free-hand serial sectioning. Dose preparations were made daily with 0.5% methylcellulose. Analysis of samples taken at the start and end of the treatment period confirmed acceptable achieved concentrations since mean results were within the range of 98-115% of nominal i.e. within the acceptable range of ±15% of nominal.	
5.2	**Results and discussion**	<u>Mortality and clinical signs</u> There were no treatment-related deaths and no clinical signs of toxicity at any dose level. <u>Bodyweight</u> (see Tables A6.8.1.1-1 and -2) At the highest dose level (20 mg/kg bw/d), bodyweight gain was reduced from the start of dosing (Day 6 pc) until Day 10 pc. Marginally lower bodyweight gain at 4 mg/kg bw/d from Day 6 pc throughout the study, was considered of doubtful biological significance. <u>Food and water consumption</u> (See Tables A6.8.1.1-3 and -4) At 20 mg/kg bw/d, food consumption was low during treatment days 6–11 *pc* but was unaffected thereafter. At 20 mg/kg bw/d, water consumption was slightly increased from Day 8 *pc* to termination.	**x**

Section A6.8.1 **Annex Point IIA, VI.6.8.1**	**Teratogenicity test** **– Rat**

		Post mortem examinations, litter data and foetal examinations (See Table A6.8.1.1-5) There were no treatment-related macroscopic findings at any dose level and no effects of maternal treatment upon litter size and survival. Litter weight and fetal weight were unaffected and there were no effects upon the incidence of malformations, skeletal or visceral anomalies, or skeletal variants. Therefore, Fipronil was not teratogenic in rats.	
5.3	**Conclusion**	Oral administration of 20 mg/kg bw/d of Fipronil to pregnant rats between Days 6 and 15 *post coitum* was maternally toxic, as evidenced by reduced bodyweight gain from Days 6 to 10 *pc* and low food consumption from Days 6 to 11 *pc*. Water consumption was also slightly increased from Days 8 to 20 *pc*. There was no effect on embryofetal development. Fipronil was not teratogenic in the rat. The maternal No Observed Adverse Effect Level (NOAEL) was 4 mg/kg bw/d. The NOEL for developmental toxicity was 20 mg/kg bw/d (the highest dose tested).	**x**
5.3.1	LO(A)EL maternal toxic effects	20 mg/kg bw/d, based on reduction in body weight gain and food consumption during the treatment period	
5.3.2	NO(A)EL maternal toxic effects	4 mg/kg/day	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	> 20 mg/kg bw/d (No adverse embryofetal effects seen up to the highest dose tested in the presence of maternal toxicity)	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	20 mg/kg bw/d (highest dose)	
5.3.5	Reliability	1	
5.3.6	Deficiencies	None	

Table A6.8.1.1-1 Group mean body weight (g)

Days *post-coitum*	Dose level (mg/kg bw/d)			
	0	**1**	**4**	**20**
Number of females	24	24	25	25
Day 2 *p.c.*	199.5	199.8	199.7	199.7
Day 6 *p.c.*	242.1	240.8	242.4	241.6
Day 8 *p.c.*	260.0	258.1	257.7	248.6
Day 10 *p.c.*	279.4	275.6	274.2	256.2
Day 12 *p.c.*	298.2	295.9	292.8	276.0
Day 14 *p.c.*	315.0	311.1	308.7	294.2
Day 16 *p.c.*	336.0	334.9	331.2	319.2
Day 20 *p.c.*	411.9	408.7	397.1	394.6

Table A6.8.1.1-2 Group mean body weight change (g)

Days *post-coitum*	Dose level (mg/kg bw/d)			
	0	**1**	**4**	**20**
Number of females	24	24	25	25
Days 6-8 *p.c.*	17.9	17.3	15.2	7.0
Days 6-10 *p.c.*	37.3	34.8	31.8	14.6
Days 6-12 *p.c.*	56.1	55.2	50.3	34.4
Days 6-14 *p.c.*	72.9	70.4	66.2	52.6
Days 6-16 *p.c.*	93.9	94.2	88.8	77.6
Days 6-20 *p.c.*	169.8	167.9	154.6	153.0

Table A6.8.1.1-3 Group mean food consumption (g/rat/day)

Days *post-coitum*	Dose level (mg/kg bw/d)			
	0	**1**	**4**	**20**
Number of females	25	25	25	25
Days 6-7 *p.c.*	28	28	28	24
Days 8-9 *p.c.*	29	29	28	22
Days 10-11 *p.c.*	31	30	30	28
Days 12-13 *p.c.*	31	30	31	30
Days 14-15 *p.c.*	32	32	31	32
Days 16-17 *p.c.*	35	33	33	35
Days 18-19 *p.c.*	34	34	33	36

Table A6.8.1.1-4 Group mean water consumption (g/rat/day)

Days *post-coitum*	Dose level (mg/kg bw/d)			
	0	**1**	**4**	**20**
Number of females	25	25	25	25
Days 6-7 *p.c.*	35	35	35	36
Days 8-9 *p.c.*	37	36	38	43
Days 10-11 *p.c.*	40	39	40	48
Days 12-13 *p.c.*	39	38	40	50
Days 14-15 *p.c.*	41	42	43	50
Days 16-17 *p.c.*	46	44	47	55
Days 18-19 *p.c.*	46	45	47	55

Table A6.8.1.1-5 Group mean litter data

	Dose level (mg/kg bw/d)			
	0	**1**	**4**	**20**
Number of females	24	24	25	25
Corpora lutea	16.1	15.8	15.9	15.7
Implantations	14.0	14.0	13.0	13.3
Pre-implantation loss (%)	14.5	11.6	19.7	15.0
Embryonic deaths (total)	0.6	0.6	1.0	0.6
Post-implantation loss (%)	3.9	5.8	7.4	5.0
Live fetuses	13.3	13.5	11.9	12.6
Litter weight (g)	52.12	52.89	45.23	49.63
Fetal weight (g)	3.96	3.98	3.82	3.96

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.1 Lot/Batch number: Batch n° *JJW 2070* , Lot n° IGB444 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.3.6 Concentration in vehicle: ~~*0.1, 0.4, 2 mg/ml*~~ 0.01, 0.04, 0.2 % (w/v)
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1 Maternal toxic effects/ 5.2 Results and discussion/ 5.3 Conclusion *Bodyweight (see Tables A6.8.1.1-1 and -2)* *At the highest dose level (20 mg/kg bw/d), bodyweight gain was reduced from the start of dosing (Day 6 pc) until* ~~*Day 10 pc*~~ the end of the study. *Marginally lower bodyweight gain at 4 mg/kg bw/d from Day 6 pc throughout the study, was considered of doubtful biological significance.*
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.1	**Teratogenicity test**
Annex Point IIA, VI.6.8.1	**– Rabbit**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.8.1/02** XXXX M&B 46030: Teratology study in the rabbit. Final Report. XXXX (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	EEC 88/302; OECD 414 (1981); EPA 83-3	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	Fine off white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	XXXX	
3.2.4	Sex	Female	
3.2.5	Age/weight at study initiation	Age: 16 – 24 weeks Weight: 3.31 – 4.82 kg	X
3.2.6	Number of animals per group	22	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Artificial insemination	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of exposure	Day 6 to day 19 of gestation	
3.3.2	Post exposure period	Day 20 to day 29 of gestation	
3.3.3	Type	gavage	
3.3.4	Concentration	0, 0.1, 0.2, 0.5 or 1.0 mg/kg bw/d	
3.3.5	Vehicle	Formulated freshly each day in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80	
3.3.6	Concentration in vehicle	0, 0.02, 0.04, 0.1, or 0.2 mg/ml	
3.3.7	Total volume applied	5 ml/kg bw	
3.3.8	Controls	Blank vehicle	
3.4	**Examinations**		
3.4.1	Body weight	Yes (daily)	

Section A6.8.1	**Teratogenicity test**
Annex Point IIA, VI.6.8.1	**– Rabbit**

3.4.2 Food consumption	Yes (days 1–5, 6–12, 13–19, 20-23 and 25–28 inclusive)	X
3.4.3 Clinical signs	Yes (daily)	
3.4.4 Examination of uterine content	Yes - Number of corpora lutea in each ovary - Number of implantation sites - Number of resorption sites (early and late - Number + distribution of live and dead fetuses in each uterine horn	
3.4.5 Examination of foetuses		
3.4.5.1 General	Number of dead and live fetuses, individual fetal weight, weight of individual placentae	
3.4.5.2 Skeletal	Yes	
3.4.5.3 Soft tissues	Yes	
3.5 Further remarks	None	X
	4. RESULTS AND DISCUSSION	
4.1 Maternal toxic effects	Mortality and clinical signs There were no treatment-related deaths and no clinical signs of toxicity at any dose level. One control female was killed *in extremis*. This death was considered incidental. Bodyweight (see Tables A6.8.1.2-1 and -2) At the highest dose levels (0.5 and 1.0 mg/kg bw/d) bodyweight gain was significantly reduced from the start of dosing (Day 6 *post coitum*, pc) throughout treatment. Marginally low bodyweight gain over the same period 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. Food consumption (see Table A6.8.1.2-3) At 0.5 and 1.0 mg/kg bw/d, food consumption was reduced from the start of treatment (Day 6 *pc*) throughout the treatment period, but was unaffected thereafter. Marginal responses seen at 0.1 and 0.2 mg/kg bw/d during the treatment period. Post mortem examination One control female and one female in each of the groups given 0.1 or 1.0 mg/kg bw/d exhibited total litter resorption (total litter loss) at necropsy (Day 29 *pc*). One female receiving 0.1 mg/kg bw/d aborted on Day 23 *pc*. These findings were considered incidental and unrelated to treatment with Fipronil.	
4.2 Teratogenic / embryotoxic effects	Litter data (see Table A6.8.1.2-4) Litter responses, as assessed by the numbers of implantations and viable young, the extent of pre- and post implantation losses and fetal and placental weights were unaffected by treatment with Fipronil. Fetal observations Examination of foetuses at necropsy, following skeletal processing or free-hand serial sectioning of foetal heads revealed a small number of findings, the majority of which were of types and occurred at incidences previously recorded in this strain of rabbit at the performing laboratories and showed no association with treatment.	
4.3 Other effects	None	X
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 Materials and	Fipronil was administered by oral gavage to groups of pregnant 22	

Section A6.8.1	**Teratogenicity test**
Annex Point IIA, VI.6.8.1	**– Rabbit**

	methods	mated New Zealand White rabbits to assess its potential to affect development of the conceptus *in utero*. The rabbits received daily Fipronil doses of either 0 (control), 0.1, 0.2, 0.5 or 1.0 mg/kg bw/d from Days 6 to 19 *post coitum* (*pc*) using a dose volume of 5 ml/kg bw. Control animals were given the vehicle, 0.5% (w/v) Methyl-cellulose and 0.5% (w/v) Tween 80. The date of insemination was designated Day 0 *pc*. The rabbits were 16-24 weeks old on arrival and were allowed at least one weeks' acclimatisation before artificial insemination. They were given 25 i.u. luteinising hormone injection (iv) approximately three weeks prior to insemination and again afterwards to ensure successful ovulation. Rabbits weighed between 3.31 to 4.82 kg at mating (Day 0 pc) and between 3.27 and 4.95 kg on the first day of dosing (Day 6 pc). Mated females were housed individually. Animals were examined daily for mortality and clinical signs of toxicity. Individual bodyweights were recorded daily and food consumption was recorded regularly throughout the study. The females were killed on Day 29 *pc* and examined macroscopically for abnormalities. The uterus of apparently not pregnant females was stained using the Salewski staining technique to reveal possible implantation sites. For pregnant females, the numbers of corpora lutea and implantations were recorded together with the type and uterine position of each implant. The weight of each live fetus and its placenta was noted. Live fetuses were killed by subcutaneous injection of pentobarbitone sodium and external abnormalities recorded. Fetuses were subsequently eviscerated and examined for visceral anomalies. Their sex was determined by inspection of the gonads. One third of the fetuses in each litter were decapitated and the heads fixed in Bouin's fluid for subsequent examination following free-hand serial sectioning. The torsos and remaining intact fetuses were fixed and subsequently stained and examined for skeletal abnormalities. Dose preparations were made daily with 0.5% (w/v) aqueous Methyl-cellulose mucilage and 0.5% (w/v) Tween 80. Samples taken during the first and last weeks of dosing were analysed for achieved concentration of Fipronil. The mean achieved values were within the range of 91-117% of the nominal dose.	X X
5.2	**Results and discussion**	<u>Mortality and clinical signs</u> There were no treatment-related deaths and no clinical signs of toxicity at any dose level. One control female was killed *in extremis*. This death was considered incidental. <u>Bodyweight</u> (see Tables A6.8.1.2-1 and -2) At the highest dose levels (0.5 and 1.0 mg/kg bw/d) bodyweight gain was significantly reduced from the start of dosing (Day 6 *post coitum*, pc) throughout treatment. Marginally low bodyweight gain over the same period 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.	

Section A6.8.1	**Teratogenicity test**
Annex Point IIA, VI.6.8.1	**– Rabbit**

		Food consumption (see Table A6.8.1.2-3) At 0.5 and 1.0 mg/kg bw/d, food consumption was reduced from the start of treatment (Day 6 *pc*) throughout the treatment period, but was unaffected thereafter. Marginal responses seen at 0.1 and 0.2 mg/kg bw/d during the treatment period. Post mortem examinations, litter data and fetal examinations (See Table A6.8.1.2-3) One control female and one female in each of the groups given 0.1 or 1.0 mg/kg bw/d exhibited total litter resorption (total litter loss) at necropsy (Day 29 *pc*). One female receiving 0.1 mg/kg bw/d aborted on Day 23 *pc*. These findings were considered incidental and unrelated to treatment with Fipronil. There were no treatment-related macroscopic findings and no effects of maternal treatment upon litter size and survival. Placental weight and fetal weight were unaffected. There were no treatment-related fetal findings at necropsy or at visceral and skeletal examination. Therefore Fipronil was not teratogenic in the rabbit.	
5.3	**Conclusion**	Oral administration of 0.5 and 1.0 mg/kg bw/d of Fipronil to pregnant rabbits from Days 6 to 19 *post coitum* was maternally toxic, as evidenced by reduced bodyweight gain and food consumption during the treatment period. There was no effect upon litter parameters or on embryofetal development. Fipronil was not teratogenic in the rabbit. The maternal NOAEL was 0.2 mg/kg bw/d. The NOEL for developmental toxicity was 1.0 mg/kg bw/d, the highest dose tested.	
5.3.1	LO(A)EL maternal toxic effects	0.5 mg/kg bw/d	
5.3.2	NO(A)EL maternal toxic effects	0.2 mg/kg bw/d	
5.3.3	LO(A)EL embryotoxic / teratogenic effects	> 1 mg/kg bw/d (no adverse effects up to the highest dose level tested)	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	≥ 0.1 mg/kg bw/d (highest dose tested)	X
5.3.5	Reliability	1	
5.3.6	Deficiencies	None	

Table A6.8.1.2-1 Group mean body weight (kg)

Days post-coitum	Dose level (mg/kg bw/d)				
	0	0.1	0.2	0.5	1.0
Number of females	19	19	21	18	18
Day 0	3.80	3.78	3.81	3.91	3.76
Day 6	3.95	3.90	3.93	4.05	3.89
Day 8	4.01	3.94	3.96*	4.08**	3.92*
Day 10	4.07	3.96***	4.00*	4.11***	3.95**
Day 12	4.10	4.00	4.00**	4.14*	3.97*
Day 14	4.17	4.06	4.06*	4.18*	4.00**
Day 16	4.24	4.11	4.12*	4.24*	4.03**
Day 18	4.25	4.14	4.14*	4.22**	4.01***
Day 20	4.25	4.12	4.16	4.20**	3.98***
Day 24	4.32	4.19	4.24	4.30*	4.06***
Day 28	4.37	4.28	4.30	4.37	4.14**

Statistical evaluation: * p≤0.05; ** p≤0.01; *** p≤0.001

X

Table A6.8.1.2-2 Group mean body weight change(kg)

Days post-coitum	Dose level (mg/kg bw/d)				
	0	0.1	0.2	0.5	1.0
Number of females	19	19	21	18	18
Days 6-8	0.06	0.04	0.03*	0.03**	0.03*
Days 6-10	~~0.12~~ 0.11	~~0.06~~ 0.05***	0.07*	0.06***	0.06**
Days 6-12	0.15	0.10	0.07**	0.09*	0.08*
Days 6-14	0.22	0.16	0.13*	0.13*	0.11**
Days 6-16	0.29	0.21	0.19*	0.19*	0.14**
Days 6-18	0.30	0.24	0.21*	~~0.17**~~ 0.17*	0.12***
Days 6-20	0.30	0.22	0.22	0.15**	0.09***
Days 6-24	0.37	0.29	0.31	0.25*	0.17***
Days 6-28	0.42	0.38	0.37	0.32	0.25**

Statistical evaluation: * p≤0.05; ** p≤0.01; *** p≤0.001

X

X

Table A6.8.1.2-3 Group mean body food consumption (g/rabbit/day)

Days *post-coitum*	Dose level (mg/kg bw/d)				
	0	0.1	0.2	0.5	1.0
Number of females	19	19	21	18	18
Days 6-12	187	171	168	180	165*
Days 13-19	184	157	166	146*	124**
Days 20-23	151	148	156	156	135
Days 24-28	132	133	131	131	130

Statistical evaluation: * p≤0.05; ** p≤0.01

Table A6.8.1.2-4 Group mean litter data

	Dose level (mg/kg bw/d)				
	0	**0.1**	**0.2**	**0.5**	**1.0**
Number of pregnant females	20	21	21	18	19
Abortion and total litter loss (%)	5.0	9.5	0.0	0.0	5.3
Corpora lutea	11.4	11.1	10.4	10.4	10.8
Implantations	10.7	9.2	9.0	9.0	9.7
Pre-implantation loss (%)	6.5	17.1	14.9	14.7	10.3
Resorptions (total)	1.8	0.9	0.9	0.9	1.1
Post-implantation loss (%)	16.7	10.3	9.5	9.9	11.4
Live fetuses	8.9	8.3	8.1	8.1	8.6
Fetal weight (g)	40.2	41.7	41.0	40.8	39.5
Placental weight (g)	5.6	5.6	5.5	5.7	5.3

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	March 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: ~~*29 November 1990*~~ 4 May 1990 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.2.5 Age/weight at study initiation/5.1 Materials and methods: ~~*Age: 16 – 24 weeks*~~ Age: 17 – 25 weeks 3.4.2 Food consumption: ~~*25-28 inclusive*~~ 24-28 inclusive 3.5 Further remarks: ~~*none*~~ Mortality: The Control female which was killed *in extremis* was subjected to a thorough macroscopic examination of the visceral organs with the object of identifying the cause of its condition. Specimens of abnormal tissues were retained.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.3 Other effects: ~~*none*~~ Terminal necropsy findings: no adverse macroscopic findings considered to be related to treatment.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: The mean achieved values were within the range of ~~*91-117%*~~ 80-125% of the nominal dose for the first week of treatment and 86-112% of the nominal dose for the last week of treatment. 5.3.4 NO(A)EL embryotoxic / teratogenic effects : ~~*≥ 0.1 mg/kg bw/d*~~ ≥ 1.0 mg/kg bw/d *(highest dose tested)*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.2 **Two generations reproduction study**	
Annex Point IIA, VI.6.8.2	

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.8.2/01** XXXX M&B 46030: Reproductive performance study in rats treated continuously through two successive generations. Final report. XXXX (unpublished) (XXXX) **A6.8.2/02** XXXX M&B 46030: Reproductive performance study in rats treated continuously through two successive generations. Amendment to final report. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	USEPA (=EPA) 83-4 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**x**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**x**
3.1.2.1	Description	Fine off-white powder	
3.1.2.2	Purity	95.4%	
3.1.2.3	Stability	Stable. Samples reanalysed every six months	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley CD	
3.2.3	Source	XXXX	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: approx 4 weeks Weight: 115 – 168 g (males) and 99 – 140 g (females)	**x**
3.2.6	Number of animals per group	30 males and 30 females	
3.2.7	Mating	One male and one female from the same treatment group avoiding pairing of siblings	
3.2.8	Duration of mating	To maximum of 21 consecutive nights	
3.2.9	Deviations from standard protocol	F_0 generation of control, low- and mid-dose groups remated to give F_{1B} offspring 10 days after previous litter was weaned For production of F_2 generation, F_{1A} litters were culled to give 30 animals per group	
3.2.10	Control animals	Yes	

Section A6.8.2	**Two generations reproduction study**
Annex Point IIA, VI.6.8.2	

3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	30 males and 30 females/group for both matings to produce F_1A and F_2 generation
3.3.2	Duration of exposure before mating	F_0 ($\rightarrow F_{1A}$): 71 days F_0 ($\rightarrow F_{1B}$): approx. 130 days F_{1A} ($\rightarrow F_2$): 71 days post-weaning (plus exposure in-utero and during lactation)
3.3.3	Duration of exposure in general P, F`1, F2 males, females	F_0: approx. 180 days (until killed when F_{1B} litter was weaned) F_{1A} approx. 120 days (from weaning until F_2 litter was weaned) F_{1B}: approx. 60 days (from weaning until F_2 litter (from F_{1A} mating) was weaned) F_2: no post-weaning exposure Not considered are pre-weaning exposures (in-utero and during lactation)
3.3.4	Type	Oral diet
3.3.5	Concentration	0, 3, 30 and 300 ppm test substance intake: see Table A6.8.2-1 food consumption per day: *ad libitum*
3.3.6	Vehicle	none
3.3.7	Concentration in vehicle	Not applicable
3.3.8	Total volume applied	Not applicable
3.3.9	Controls	Blank diet
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Body weight	Yes
3.4.3	Food/water consumption	Food: yes Water: no
3.4.4	Oestrus cycle	yes
3.4.5	Sperm parameters	testis weight epididymides weight Sperm content and morphology (histopathology of testes and epididymides)
3.4.6	Offspring	number and sex of pups stillbirths live births presence of gross anomalies weight gain physical or behavioural abnormalities Physical development

Section A6.8.2 **Annex Point IIA, VI.6.8.2**	**Two generations reproduction study**	
3.4.7 Organ weights P and F1	Uterus ovaries testes epididymides prostate seminal vesicles liver pituitary thyroid vagina mammary glands	
3.4.8 Histopathology P and F1	Vagina uterus ovaries testis epididymis prostate thyroid Liver pituitary kidney	**x**
3.4.9 Histopathology F1 not selected for mating, F2	As above	
3.5 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Effects		
4.1.1 Parent males	No effects on mortality (no indication that two deaths at 300 ppm, and 1 humane kill at 30 ppm were treatment related). No clinical signs. 300 ppm: Transiently decreased body weight gain (weeks 10-19); decreased food intake No effects on mating performance and fertility 300 ppm: decreased terminal body weight, increased liver weight 30 ppm: slight rel. liver weight increase 30 + 300 ppm: increased thyroid weight 30 + 300 ppm: increased follicular cell hypertrophy (2/29n.s., 10/29***)	
4.1.2 Parent females	300 ppm: 5 deaths at 300 ppm (3 found dead; 1 died and 1 was killed after convulsions, salivation, limited use of hind limb and signs of general toxicity); two further females with convulsions 300 ppm: Decreased body weight gain during mating, gestation and lactation; decreased food intake 300 ppm: Reduced terminal body weight, pituitary, ovary weight; increased liver and thyroid weight 30 ppm: increased liver weight 300 ppm: centriacinar hepatocytic fatty vacuolation (9/26***) 300 ppm: increased follicular cell hypertrophy (6/26**)	

Section A6.8.2 **Annex Point IIA, VI.6.8.2**	**Two generations reproduction study**	
4.1.3 F_1 males	Offspring toxicity (up to weaning): see point 4.2 below 300 ppm: decreased body weight upon start and end of 19-week treatment; reduced body weight gain; reduced food intake 300 ppm: slightly decreased mating and fertility index 300 ppm: decreased terminal body weight 30 and 300 ppm: increased liver and thyroid weight 30 + 300 ppm: increased follicular cell hypertrophy (3/30n.s., 9/30**)	**X**
4.1.4 F_1 females	Offspring toxicity (up to weaning): see point 4.2 below 300 ppm: One female with convulsions on Day 20 *p.c.* 300 ppm: reduced bw upon start of treatment, reduced bw during gestation and lactation 300 ppm: slightly increased incidence of irregular estrous cycle within background control range 300 ppm: slightly decreased mating and fertility index 30 + 300 ppm: inceased liver and thyroid weight 30+300 ppm: decreased pituitary weight 300 ppm: centriacinar hepatocytic fatty vacuolation (6/27*) 30 + 300 ppm: increased follicular cell hypertrophy (7/29*, 15/27***)	**X**
4.1.5 F_2 males	Offspring toxicity (up to weaning): see point 4.2 below	
4.1.6 F_2 females	Offspring toxicity (up to weaning): see point 4.2 below	
4.2 Other	<u>Litter parameters and pup toxicity:</u> **F_{1A} pups:** 300 ppm: decreased live birth index and viability index; no effect during lactation. Decreased litter size before litter size adjustment on day 4 p.p. 300 ppm: Decreased birth weights and decreased pup weight gain during lactation 300 ppm: Slightly delayed onset of tooth eruption 300 ppm: Convulsions in 14 pups from 9 litters during lactation days 14-20 **F_{1B} pups**: No effects up to 30 ppm (highest dose tested) **F_2 pups:** 300 ppm: Convulsions in 4 F2 pups from 3 litters on Day 15 p.p. and in one F2 pup on Day 18 p.p. 300 ppm: decreased post-implantation survival, live birth and viability indices 300 ppm: Reduced mean pup weight as determined before litter adjustment and on Day 25 p.p. 300 ppm: Marginal delay in onset of pinna unfolding	**X**
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** In a two-generation reproduction toxicity study, groups of 30 male and 30 female F_0 Sprague-Dawley CD rats were treated with 0, 3, 30 or 300 ppm of Fipronil and used to derive a second generation of similar group sizes and fed the same dietary concentrations. Two litters were produced from the F_0 generation and one from the F_1 generation, which were assessed for potential effects on reproductive capability as well as viability, growth and development of the pups. At 300 ppm, one male and one female from the F_0 generation was replaced during	

Section A6.8.2 **Annex Point IIA, VI.6.8.2**	**Two generations reproduction study**	
	the first week of treatment due to the death and cannibalisation of the original animals. The parental F_0 generation animals were littermates, and approximately 4 weeks old at arrival. They were allowed approximately 2 weeks acclimatisation before treatment commenced and weighed 115 to 168 g (males) and 99 to 140 g (females) at the start of treatment. Rats were fed for 71 days before pairing one male with one female until mating occurred (confirmed by the presence of sperm in the vaginal smear), or for a maximum of 21 consecutive nights. Vaginal smears were also taken for 10 days prior to pairing for production of the F_1A litters to establish the estrus status normality or otherwise of estrus. F_0 generation animals reared their F_1A offspring to weaning (Day 25 *post partum*), during which time offspring survival and growth and developmental landmarks (pinna unfolding, hair growth, tooth eruption and eye opening) were monitored. Litter size was adjusted to a maximum of 8 offspring (4 male and 4 female offspring, where possible) by random culling on Day 4 *post partum* to standardise the litter parameters. The culled offspring were examined macroscopically internally and externally for macroscopic abnormalities. At weaning (Day 25 *post partum*), groups of 30 male and 30 female F_1A offspring were selected to form the F_1 generation. Surplus F_1A offspring were killed and examined for macroscopic abnormalities. Shortly after weaning of the F_1A litters, the F_0 generation animals from the groups fed 0, 3 and 30 ppm of Fipronil were remated to produce the F_1B litters, which were raised to weaning (Day 25 *post partum*). F_0 animals fed 300 ppm were not remated, but were maintained on treated diet until the F_1B litters were weaned and then all the F_0 animals were necropsied. The F_1B offspring were killed after weaning and examined macroscopically. The selected F_1 generation was treated for 71 days before pairing one male with one female until mating occurred (confirmed by the presence of sperm in the vaginal smear), or for a maximum of 21 consecutive nights. Vaginal smears were also taken for 10 days prior to pairing to establish the normality or otherwise of estrus. The resultant F_2 offspring were reared to weaning (Day 25 *post partum, pp*), during which time their survival, growth and developmental landmarks were monitored (as for the F1 litters). Litters were culled to 8 offspring on Day 4 *pp*. All parental animals (F_0 and F_1) were subject to a detailed necropsy after weaning of their offspring. The gonads, accessory reproductive organs, liver and thyroid were weighed and histopathological examinations performed on the control and high dose level animals. The liver and thyroid from the low and intermediate dose groups were also examined. All macroscopic abnormalities were evaluated microscopically. Parameters monitored for the F_0 and F_1 parental animals included: daily observations for clinical signs and mortality, bodyweight of males (recorded weekly to termination) and females (weekly prior to pairing and on Days 0, 6, 13 and 20 *post coitum, pc*, and on Days 1, 4, 7, 14, 21 and 25 *pp*),	**x**

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	vaginal smears for 10 days prior to pairing to produce F_1A and F_2 litters and during pairing onset and completion of parturition, macroscopic observations at necropsy, histopathological examinations of the reproductive and target (liver and thyroid) organs of control and high dose animals. Target organs and abnormalities from other groups; abnormalities from all rats. Parameters monitored for litters included: observations for mortality and clinical signs daily from about 24 hours after birth number of live and dead pups at birth, sex ratio at Day 1 (approx. 24 hours after birth) and on Days 4 (before and after culling), 7, 14, 21 and 25 *pp*), individual body weight at Day 1 (approx. 24 hours after birth) and on Days 4 (prior to culling), 7, 14, 21 and 25 *pp*, physical development (pinna unfolding, hair growth, tooth eruption and eye opening) macroscopic observations (culled and surplus offspring) at termination; tissue retention (reproductive organs, liver and thyroid) from unselected F_1A offspring, F_2A offspring and, where possible, from 1 male and 1 female pup from F_1B litters. Test diets were prepared freshly each week. Prior to the start of treatment, homogeneity of mixing and stability following 31 days storage at 21^0C of Fipronil was evaluated in trial mixes. Homogeneity was confirmed at concentrations of 3 and 300 ppm of Fipronil and stability at concentrations of 1 and 500 ppm. Analysis of the test diet samples taken during the first four weeks of the treatment and at monthly intervals thereafter until termination was performed to establish the achieved concentrations of Fipronil. Achieved values were 97.9%, 92.2% and 93.4% of nominal at 3, 30 and 300 ppm, respectively i.e. were within acceptable limits.	**x**
5.2 Results and discussion	**Parental toxicity, F_0 generation** <u>Mortality and signs</u> There were seven deaths observed at 300 ppm (2 males and 5 females): One male and one female were found dead during the first week of treatment. Both were severely cannibalised and were replaced. One male was found dead during Week 24, one female, severely cannibalised, was found dead on Day 17 post coitum (p.c). One female died and another female was killed for humane reasons following convulsion and exhibiting excessive salivation, pallor, rregular or gasping respiration and limited use of hind-limbs on Day 14 post partum (p.p.). Two other females were found dead on Day 17 *p.c.* and Day 17 p.p., respectively. No treatment-related findings were seen at necropsy or upon histopathological examination of any of these animals. At 30 ppm one male was killed for humane reasons during Week 23 with decreased muscular control and limited use of the hind limbs. This death was considered incidental. One control female was killed *in extremis* during lactation following a general deterioration in condition.	**x**

Section A6.8.2 **Annex Point IIA, VI.6.8.2**	**Two generations reproduction study**	
	Beside the two females that died or were killed after convulsions, two further females fed 300 ppm exhibited single episode of convulsions and survived to scheduled sacrifice. Bodyweight (see Tables A6.8.2-2 and -3) At 300 ppm, male bodyweight gain was reduced compared with controls between Weeks 10 and 19 and throughout the study in females, including during gestation and lactation. At 30 ppm, male bodyweight gain was slightly low during the first week of treatment. No other treatment-related effects were seen. Food consumption (see Table A6.8.2-4) At 300 ppm food consumption was lower than controls in both sexes. Food conversion efficiency was significantly low in Week 1 (males: 25.0 compared with 37.0 for controls; females: 21.3 compared with 26.3 in controls). Mating performance, pregnancy rate and littering Estrous cycles, mating performance and fertility and gestation length were unaffected by treatment. Macroscopic examination No effects Organ weights: (see Table A6.8.2-15) Absolute and body weight relative liver and thyroid weights were increased in both sexes at 30 and 300 ppm whilst ovarian weights were reduced at 300 ppm. Histopathology: (see Table A6.8.2-16) There was an increased incidence of centriacinar fatty vacuolation in the liver of females fed 300 ppm. Furthermore, follicular epithelial hypertrophy was increased in the thyroid of males given 30 ppm and in both sexes fed 300 ppm. **Parental toxicity, F_1 generation** Mortality and signs At 30 and 300 ppm, one female was killed for humane reasons during parturition following prolonged vaginal bleeding. Neither mortality was considered to be treatment-related. One control female was killed *in extremis* during gestation with trauma of the buccal cavity. This death was also incidental. At 300 ppm, there were two other female deaths. One female was found dead on Day 16 *post partum* with matted hair around the mouth and nares. The other one was killed for humane reasons with oedema of the head and mutilation of the digits. Previously it had exhibited a short convulsion. No treatment-related findings were evident at necropsy or histopathological examination. Bodyweight (see Tables A6.8.2-5 and -6) At 300 ppm, bodyweight of males and females at selection of the F_1 generation was lower than controls. Subsequently weight gain in males was also reduced up to Week 19. Although weight gain of females prior to pairing was unaffected, it was reduced during gestation and lactation. Food consumption (see Table A6.8.2-7) At 300 ppm, food consumption in males was lower than in controls. Their food conversion efficiency was also slightly low. Consumption and food efficiencies in all other groups were similar to controls.	

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	Mating performance, pregnancy rate and littering (see Table A6.8.2-8) Estrous cycles and gestation length were unaffected by treatment. However, the percentage of animals that mated and consequently, the fertility index, were slightly low at 300 ppm. Macroscopic examination No effects Organ weights: (see Table A6.8.2-17) Absolute and bodyweight relative liver and thyroid weights were increased in both sexes at 30 and 300 ppm whilst absolute pituitary weights were significantly lower than controls in females fed 30 and 300 ppm. Although absolute ovary, testis and epididymal weights were lower at 300 ppm, their weights relative to body weight were unaffected, apart from the testis, which was higher. Histopathology: (see Table A6.8.2-18) As in the F_0 animals, there was an increased incidence of centriacinar fatty vacuolation in the liver of females given 300 ppm. Furthermore, the incidence of thyroid follicular epithelial hypertrophy was increased in both sexes at 30 ppm (10/59 rats) and 300 ppm (24/57 rats). A marginal thyroid response in two males fed 3 ppm was considered to be within the normal range since it is well known that in the rat this organ is very sensitive to stimulus. **Effects on litter parameters, pre-weaning and post-weaning development** - F_0 to F_1A litters Litter size, pup loss and litter and mean pup weights (see Tables A6.8.2-9 and -10) At 300 ppm, thirteen offspring in nine litters showed convulsions between Days 14 and 20 *post partum* (i.e. at the time the offspring would be starting to eat the diet). Also at 300 ppm, litter size, the live birth index and viability index at Day 4 of age prior to culling were reduced. The sex ratio was unaffected. At the 300 ppm high dose level, bodyweights of F_1A offspring at birth and their weight gain up to weaning were significantly lower than controls. Pre-weaning development (see Table A6.8.2-11) At 300 ppm, the only finding was a slight delay in the onset of tooth eruption. There were no other treatment-related changes. **Effects on litter parameters, pre-weaning and post-weaning development** - F_0 to F_1B litters No treatment-related effects on any parameter were reported on these litters killed at weaning (Day 25 *post partum*). **Effects on litter parameters, pre-weaning and post-weaning development** - F_1A to F_2 litters	**X**

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	Litter size, pup loss and litter and mean pup weights (see Tables A6.8.2-12 and -13) At 300 ppm, five offspring in four F_2 litters had convulsions on Day 15 or 18 *post partum* (i.e. at the time when they were expected to start eating diet). Also at 300 ppm, post-implantation survival of the conceptus and post-natal viability of the pups to Day 4 *post partum* were significantly reduced. However, the sex ratio was unaffected. At 300 ppm, bodyweights of the F_2 offspring at birth and weight gain up to weaning were significantly lower than controls. Pre-weaning development (see Table A6.8.2-14) At 300 ppm, there was a slight delay in pinna unfolding. No other treatment-related changes were seen.	
5.3 Conclusion	Continuous dietary administration of 300 ppm of Fipronil to rats for two successive generations was toxic to adults, as evidenced by mortality, convulsions and a reduction in bodyweight gain and food consumption. Mating performance of the F_0 generation was unaffected, but was slightly reduced in the F_1 generation was with a consequent reduction in fertility index. Hepatic centriacinar fatty vacuolation was seen in both generations of females and thyroid follicular hypertrophy in both sexes of the F_0 and F_1 generations. Both histopathological findings were associated with increased liver and thyroid weights. Pituitary weights were low in F_1 females. With respect to reproduction parameters, treatment with 300 ppm of Fipronil was toxic to the neonates and slightly reduced pre-implantation survival. Reductions in post-natal pup viability and weight gain occurred in both F_1A and F_2 litters as well as a slight delay in some developmental milestones (tooth eruption in F_1A litters and pinna unfolding in F_2 litters). Overt toxicity was manifest as convulsions in some offspring when they first started to consume the diet. Treatment with 30 ppm of Fipronil increased liver and thyroid weights in F_0 and F_1 animals whilst absolute pituitary weight was low in F_1 females. Thyroid weight changes were associated with follicular epithelial hypertrophy in F_1 adults and F_0 males.	
5.3.1 LO(A)EL	General toxicity: 30 ppm, based on increased liver and thyroid weight and increased follicular cell hypertrophy of the thyroid in both parental generations and decreased pituitary weight in F1 females without histopathological correlate Reproduction toxicity: 300 ppm based on decreased mating and fertility indices in F_1 generation Offspring toxicity: 300 ppm, based on reduced post-implantation survival, live birth and viability indices, reduced pup weight development, convulsions during lactation days 14-18, and slight delay in onset of tooth eruption (F_1 pups) and in onset of pinna unfolding (F_2 pups)	
5.3.1.1 Parent males	General toxicity: 30 ppm Reproduction toxicity: > 300 ppm	

Section A6.8.2	**Two generations reproduction study**	
Annex Point IIA, VI.6.8.2		
5.3.1.2 Parent females	General toxicity: 30 ppm Reproduction toxicity: > 300 ppm	
5.3.1.3 F1 males	General toxicity: 30 ppm Reproduction toxicity: 300 ppm Offspring toxicity: 300 ppm	
5.3.1.4 F1 females	General toxicity: 30 ppm Reproduction toxicity: 300 ppm Offspring toxicity: 300 ppm	
5.3.1.5 F2 males	Offspring toxicity: 300 ppm	
5.3.1.6 F2 females	Offspring toxicity: 300 ppm	
5.3.2 NO(A)EL	General toxicity: 3 ppm (equivalent to 0.25 mg/kg bw/d in males and 0.27 mg/kg bw/d in females), based on increased liver and thyroid weight and increased follicular cell hypertrophy of the thyroid in both parental generations and decreased pituitary weight in F1 females without histopathological correlate at 30 ppm Reproduction toxicity: 30 ppm (equivalent to 2.53 mg/kg bw/d for males or 2.74 mg/kg bw/d for females) based on decreased mating and fertility indices in F_1 generation at 300 ppm Offspring toxicity: 30 ppm (equivalent to 2.53 mg/kg bw/d for males or 2.74 mg/kg bw/d for females), based on reduced post-implantation survival, live birth and viability indices, reduced pup weight development, convulsions during lactation days 14-18, and slight delay in onset of tooth eruption (F_1 pups) and in onset of pinna unfolding (F_2 pups) at 300 ppm.	
5.3.2.1 Parent males	General toxicity: 3 ppm Reproduction toxicity: ≥ 300 ppm	
5.3.2.2 Parent females	General toxicity: 3 ppm Reproduction toxicity: ≥ 300 ppm	
5.3.2.3 F_1 males	General toxicity: 3 ppm Reproduction toxicity: 30 ppm Offspring toxicity: 30 ppm	

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5.3.2.4 F_1 females	General toxicity: 3 ppm Reproduction toxicity: 30 ppm Offspring toxicity: 30 ppm
5.3.2.5 F_2 males	Offspring toxicity: 30 ppm
5.3.2.6 F_2 females	Offspring toxicity: 30 ppm
5.3.3 Reliability	1
5.3.4 Deficiencies	None

Table A6.8.2-1 Group mean test substance intake before pairing (mg/kg bw/d)

		Dose level, ppm		
		3 ppm	**30 ppm**	**300 ppm**
F_0	Male	0.25	2.54	24.74
	Female	0.28	2.77	27.51
F_1	Male	0.24	2.54	27.32
	Female	0.26	2.71	29.28
Combined generations	Male	0.25	2.54	26.03
	Female	0.27	2.74	28.40

Table A6.8.2-2 Group mean bodyweights for F_0 generation (g)

Week of treatment	**Dose level (ppm)**							
	0	**3**	**30**	**300**	**0**	**3**	**30**	**300**
	Bodyweight				**Bodyweight change from Week 0**			
Males								
0	138	137	135	140	-	-	-	-
1	205	203	197	172	67	66	62***	32***
5	424	416	404	381	286	279	269	241
10	548	536	530	512	410	399	395	372**
15	634	617	608	584	496	480	473	444***
20	676	658	649	632	538	521	514	492
26	740	721	705	687	602	584	570	547
Females prior to pairing								
0	121	121	118	117	-	-	-	-
1	161	158**	155**	140***	40	37**	37**	23***
5	260	256	252	239	139	135	134	122
10	312	303	304	290***	191	182	186	173***

** p≤0.01 *** p≤0.001

X

Table A6.8.2-3 Group mean bodyweights of F_0 females during gestation and lactation

Days	Dose level (ppm)							
	0	3	30	300	0	3	30	300
During gestation of F_1A litters								
Day pc	Bodyweight				Bodyweight change from Day 0 pc			
0	307	306	304	290*	-	-	-	-
6	340	339	337	315	33	33	33	25
13	378	377	373	349	71	71	69	59
20	463	458	458	418***	156	152	154	128***
During lactation of F_1A litters								
Day pp	Bodyweight				Bodyweight change from Day 1 pp			
1	352	357	354	323**	-	-	-	-
4	357	363	357	325	5	6	3	2
7	365	366	365	329	13	9	11	6
14	381	384	382	338	29	27	28	15
25	362	356	353	329***	10	-1	-1	6

Statistical evaluation: * p<0.05 ** p<0.01 *** p<0.001

X

Table A6.8.2-4 Group mean food consumption for F_0 generation prior to pairing (g/rat/week)

Week of treatment	Dose level (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
1	182	183	174	124***	154	152	145*	109***
2	199	193	192	171	155	151	149	139
3	213	212	204	196	157	157	150	144
4	213	213	208	201	150	147	144	139
5	211	211	209	200	150	147	144	141
6	211	207	207	198	150	147	143	139
7	208	204	207	199	149	146	145	142
8	208	206	207	202	151	144	144	144
9	202	202	204	196	146	143	141	137
10	208	203	205	199***	145	141	141	139**
Total[#] (% control)	2055	2034 (99)	2017 (98)	1886 (92)	1507	1475 (98)	1446 (96)	1373 (91)

[#] Weeks 1-10

Statistical evaluation: * p≤0.05 ** p≤0.01 *** p≤0.001

Table A6.8.2-5 Group mean bodyweight of F_1 generation (g)

Weeks of treatment	Dose level (ppm)							
	0	3	30	300	0	3	30	300
	Bodyweight				Bodyweight change from Week 0			
Males								
0	146	144	139	114***				
1	215	213	207	172	69	69	68	58
5	458	442	449	390	312	298	310	276
10	585	571	572	504***	439	427	433	390***
19	704	686	685	605***	558	542	546	491***
Females prior to pairing								
0	128	133	122	106***				
1	170	178	165	146	42	45	43	40
5	261	280	264	247	133	147	142	141
10	314	327	319	303	186	194	197	197

Statistical evaluation: *** $p \leq 0.001$

X

Table A6.8.2-6 Group mean bodyweight of F_1 females during gestation and lactation (g)

	Dose level (ppm)							
	0	3	30	300	0	3	30	300
During gestation of F_2 litters								
Day pc	Bodyweight				Bodyweight change from Day 0			
0	322	332	321	299*				
6	358	367	358	327	36	35	37	28
13	397	406	397	367	75	74	76	68
20	474	482	478	441*	152	150	157	142
During lactation of F_2 litters								
Day pp	Bodyweight				Bodyweight change from Day 1			
1	378	390	373	332**				
4	383	391	376	339	5	1	3	7
7	385	395	375	341	7	5	2	9
14	401	406	389	337	23	16	16	5
25	377	375	361	327***	-1	-15	-12	-5

Statistical evaluation: * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.001$

Table A6.8.2-7 Group mean food consumption for F_1 generation prior to pairing (g/rat/week)

Weeks of treatment	Dose level (ppm)							
	0	3	30	300	0	3	30	300
	Males				Females			
1	165	168	167	152*	133	143	136	132
2	204	201	204	186	148	151	148	145
3	219	217	217	201	146	153	151	147
4	228	220	230	211	140	150	146	146
5	237	230	244	229	145	154	152	150
6	237	232	243	228	144	149	149	152
7	222	218	231	211	143	150	151	152
8	224	217	232	213	149	156	156	156
9	215	206	219	203	147	146	150	155
10	224	211	223	205*	149	153	153	161
Total[#]	2175	2120	2210	2039	1444	1505	1492	1496
(% of control)		(97)	(102)	(94)		(104)	(103)	(104)

Weeks 1-10; Statistical evaluation: * = p< 0.05 (t-test following 1-way analysis of variance)

Table A6.8.2-8 Mating performance of F_1 animals

	Dose level (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
Number paired	30	30	30	30	30	30	30	30
Number mating	30	29	29	25	30	29	30	25
Mating index (%)	100	97	97	83*	100	97	100	83*
Fertility index (%)	90	97	90	80	90	97	93	80

Statistical evaluation: * p≤0.05

Table A6.8.2-9 Group mean litter size and viability of F_{1A} offspring

	Dose level (ppm)			
	0	3	30	300
Total pups at Day 1 pp	14.8	14.0	14.1	12.1***
Live pups at Day 1 pp	14.5	13.7	14.1	10.0***
Live pups at Day 4 pp	14.0	13.5	13.7	9.6***
Live pups at Day 4 pp (after cull)	7.8	8.0	8.0	7.4
Live pups at Day 25 pp	7.8	7.9	7.9	7.2
Live birth index (%)	98	98	100	83**
Viability index at Day 4 pp (%)	97	99	97	89*
Lactation index at Day 25 pp (%)	100	99	98	97

* p≤0.05 ** p≤0.01 *** p≤0.001

Table A6.8.2-10 Group mean bodyweight and body weight gain of F_{1A} offspring (g)

Day *post partum* (pp)	Dose level (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
1[a]	6.7	6.8	6.5	6.3*	6.3	6.5	6.1	5.9*
4[a]	9.9	10.2	9.7	8.8**	9.3	9.7	9.2	8.3***
25	90.1	90.2	86.7	69.9***	84.4	85.1	81.9	66.3***
Weight gain Days 1-4 pp	3.2	3.4	3.2	2.5	3.0	3.2	3.1	2.4
Weight gain Days 1-25 pp	83.4	83.4	80.2	63.6***	78.1	78.6	75.8	60.4***

[a] Weights prior to culling
* p≤0.05; ** p≤0.01; *** p≤0.001

Table A6.8.2-11 Tooth eruption of F_{1A} offspring (Day post partum)

		Dose level (ppm)			
		0	3	30	300
Tooth eruption	Onset	9.7	9.4	9.7	10.4*
	Completion	11.7	11.6	11.6	12.0

* p<0.05

Table A6.8.2-12 Group mean litter size and viability of F_2 offspring

	Dose level (ppm)			
	0	3	30	300
Implantation sites	14.9	14.1	15.1	14.6
Total pups at Day 1 pp	13.6	11.9	13.4	11.8
Live pups at Day 1 pp	13.6	12.5	13.3	10.5**
Live pups at Day 4 pp	13.3	12.3	13.0	10.7
Live pups at Day 4 pp (after cull)	7.7	7.7	7.9	7.3
Live pups at Day 25 pp	7.7	7.4	7.8	7.2
Post implantation survival (%)	90	84*	85	81*
Live birth index (%)	100	98	99	78***
Viability index at Day 4 pp (%)	98	99	98	73***
Lactation index at Day 25 pp (%)	100	97	98	92

Statistical evaluation: * p<0.05 ** p<0.01 *** p<0.001

Table A6.8.2-13 Group mean bodyweight and body weight gain of F_2 offspring (g)

Day *post partum* (pp)	Dose level (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
1[a]	6.5	6.7	6.5	6.0**	6.1	6.3	6.1	5.5***
4[a]	9.8	10.3	9.9	8.6*	9.1	9.7	9.2	7.8*
25	84.3	88.1	84.7	66.7***	79.3	83.2	79.6	62.0***
Weight gain Days 1-4 pp	3.3	3.6	3.4	2.6	3.0	3.4	3.1	2.3
Weight gain Days 1-25 pp	77.8	81.4	78.2	60.7***	73.2	76.9	73.5	56.5***

[a] Weights prior to culling
* p≤0.05; ** p≤0.01; *** p≤0.001

Table A6.8.2-14 Pinna unfolding of F_2 offspring (day of age)

		Dose level (ppm)			
		0	3	30	300
Pinna unfolding	Onset	2.7	2.5	2.7	3.0
	Completion	3.3	3.3	3.5	3.8

Table A6.8.2-15 Group mean organ weights of F_0 animals

	Males				Females			
	0 ppm	3 ppm	30 ppm	300 ppm	0 ppm	3 ppm	30 ppm	300 ppm
Bodyweight (g)	736.4	716.1	698.1	677.7*	392.1	383.8	389.8	352.0**
Absolute weights (g)								
Liver	24.8	24.3	25.5	34.0**	16.4	15.6	17.8*	19.1**
Thyroids	0.029	0.029	0.033*	0.044**	0.026	0.023	0.030	0.036**
Ovaries	N/A	N/A	N/A	N/A	0.126	0.132	0.131	0.090**
Bodyweight – relative values (%)								
Liver	3.36	3.38	3.66*	5.00**	4.19	4.07	4.57**	5.45**
Thyroids	0.0040	0.0040	0.0048*	0.0064**	0.0066	0.0062	0.0078	0.0103**
Ovaries	N/A	N/A	N/A	N/A	0.0324	0.0345	0.0335	0.0255**

N/A not applicable
Statistical evaluation: * p≤0.05; ** p≤0.01

Table A6.8.2-16 Group incidence of histopathological findings of F_0 animals

Finding	Dose level (ppm)							
	Males				Females			
	0	3	30	300	0	3	30	300
Number of rats examined	30	30	29	29	29	30	30	26
Liver								
Centriacinar hepatocytic fatty vacuolation	0	0	0	0	0	0	0	9***
Thyroid								
Hypertrophy of follicular epithelium	0	0	2	10***	0	0	0	6**

Statistical evaluation: ** p≤0.01; *** p≤0.001

Table A6.8.2-17 Group mean organ weights F_1 animals

	Males				Females			
	0 ppm	**3 ppm**	**30 ppm**	**300 ppm**	**0 ppm**	**3 ppm**	**30 ppm**	**300 ppm**
Bodyweight (g)	694.1	674.8	674.7	594.8**	369.8	385.1	367.8	358.3
Absolute weights (g)								
Liver	24.0	23.1	27.1**	28.8**	14.0	14.4	15.5**	19.8**
Thyroids	0.030	0.030	0.038**	0.044**	0.023	0.024	0.027*	0.033**
Pituitary	0.012	0.011	0.011	0.010	0.016	0.015	0.013**	0.012**
Testis	3.96	3.89	3.99	3.70**	N/A	N/A	N/A	N/A
Epididymis	1.576	1.504	1.507	1.445*	N/A	N/A	N/A	N/A
Ovaries	N/A	N/A	N/A	N/A	0.134	0.128	0.131	0.115*
Bodyweight – relative values (%)								
Liver	3.46	3.42	4.01**	4.82**	3.79	3.74	4.23**	5.52**
Thyroids	0.0044	0.0044	0.0057**	0.0074**	0.0064	0.0062	0.0075*	0.0094**
Pituitary	0.0017	0.0017	0.0016	0.0018	0.0045	0.0039*	0.0036**	0.0035**
Testis	0.576	0.579	0.598	0.628*	N/A	N/A	N/A	N/A
Epididymides	0.2287	0.2243	0.2266	0.2451	N/A	N/A	N/A	N/A
Ovaries	N/A	N/A	N/A	N/A	0.0364	0.0333	0.0359	0.0330

Statistical evalulation: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; N/A = not applicable

Table A6.8.2-18 Group incidence of pathology findings of F_1 animals

	Males				Females			
	0 ppm	**3 ppm**	**30 ppm**	**300 ppm**	**0 ppm**	**3 ppm**	**30 ppm**	**300 ppm**
Liver								
Number examined	30	30	30	30	29	30	29	27
Centriacinar hepatocytic fatty vacuolation	0	0	0	0	1	1	2	6*
Thyroids								
Number examined	28	30	30	30	29	30	29	27
Hypertrophy of follicular epithelium	0	2	3	9**	0	0	7*	15***

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.2.5 Age/weight at study initiation: *Age:* ~~*approx 4 weeks*~~ approx 5 to 6 weeks. 3.4.8 Histopathology P and F1: + lymphs nodes, lungs, skin and thymus.
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.1.3 F_1 males: 3 + *30 + 300 ppm: increased follicular cell hypertrophy (3/30n.s., 9/30**)* 300 ppm: Absolute epididymis and testes weight were, but except for testes, relative weight was not statistically significant different. 4.1.4 F_1 females: One female in each of groups 30 and 300 ppm was killed for humane reasons during parturition. One female at 300 ppm was found dead on day 16 pp. Another female was killed for humane reasons showing convulsion, oedema of the head and mutilation of the digits. 300 ppm: Absolute ovary weight was lower but relative weight was not statistically significant different.. 4.2 Other: *F_{1A} pups 300 ppm: Convulsions in* ~~*14*~~ 13 *pups from 9 litters during lactation days 14-20* *F_2 pups:* Post-implantation survival was also slightly reduced in groups 3 and 30 ppm, but post-natal viability was unaffected.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Shortly after weaning of the F_1A litters* (10 days), *sex ratio at Day 1 (approx. 24 hours after birth) and on Days 4 (before and after culling),* ~~*7,*~~ *14,* ~~*21*~~ *and 25 pp),* 5.2 Results and discussion: *Parental toxicity, F_0 generation* *Mortality and signs:* ~~*one female, severely cannibalised, was found dead on Day 17 post coitum (p.c).*~~ *Two other females were found dead on Day 17 p.c.* severely cannibalised, *and Day 17 p.p., respectively*. One control female was killed in extremis on day 4 pp, following significant weight loss and terminal signs of underactivity, piloerection, hunched posture, laboured respiration and unsteady gait. *Effects on litter parameters, pre-weaning and post-weaning development* *- F_0 to F_1A litters Litter size, pup loss and litter and mean pup weights:* *Also at 300 ppm, litter size, the live birth index and viability index at* ~~*Day 4*~~ Day 1
Reliability	1
Acceptability	acceptable
Remarks	

COMMENTS FROM …
Date
Results and discussion
Conclusion
Reliability
Acceptability
Remarks

Section A6.9 **Annex Point IIIA, VI.1**	**Neurotoxicity study**

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (1st study)**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.9/01** XXXX M&B 46030: Single Exposure Peroral (Gavage) Neurotoxicity Study in Sprague Dawley Rats. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes EPA 81-8	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	78/GC/90	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	White powder	
3.1.2.2	Purity	96.7%	
3.1.2.3	Stability	Stable	X
3.2	**Reference Substance (positive control)**	None included in this study. Observers were trained in regular proficiency test sessions (listed tests from 1988-1993) with a variety of positive control compounds including Chlorpromazine (leg splay, decreased startle response), amphetamine, (stereotypy, piloerection), acrylamide (limb weakness), and DDT (tremor).	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Sprague-Dawley	
3.3.3	Source	XXXX	
3.3.4	Sex	Male and female	
3.3.5	Rearing conditions	Normal animal husbandry	
3.3.6	Age/weight at study initiation	Approx. 7 weeks Body weight range: males 221.8 to 291.0g; females 144.5 to 186.3g	
3.3.7	Number of animals per group	15 male and 15 female	
3.3.8	Control animals	15 male and 15 female	
3.4	**Administration**	Oral	
3.4.1	Exposure	Single dose by gavage	
3.4.2	Dose levels	0, 0.5, 5.0, or 50.0 mg/kg bw	
3.4.3	Vehicle	Corn oil	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (1st study)**	
3.4.4 Concentration in vehicle	0, 0.05, 0.5 or 5 mg/ml	
3.4.5 Total volume applied	10 ml/kg bw	
3.4.6 Postexposure period	16 days	
3.4.7 Anticholinergic substances used	Not applicable (not an OP)	
3.4.8 Controls	Vehicle	
3.5 Examinations		
3.5.1 Body weight	Yes On the day prior to dosing, 7-day and 14-day post dosing	
3.5.2 Signs of toxicity	Yes <u>Mortality and overt clinical signs:</u> twice daily, 7 days/week <u>Functional observational battery (FOB):</u> 1 week prior to dosing; 7 h, 7 days and 14 days post-dosing <u>Motor activity evaluations</u> Subsequent to FOB evaluations, 1 week prior to dosing; 7 h, 7 days and 14 days post-dosing. All test sessions were 90 min in duration with 10-min intermissions	
3.5.3 Observation schedule	See above	
3.5.4 Clinical chemistry	No	
3.5.5 Pathology	Yes All animals 15 days after treatment (Day 16)	
3.5.6 Histopathology	Yes 15 days after treatment (Day 16), in-situ intracardiac perfusion under pentobarbitol anaesthesia was performed on 10 rats/group/sex Tissues were processed and evaluated for 6 rats/sex for the high-dose and control groups. Tissues: Forebrain, Cerebrum, center Midbrain, center Medulla oblongata Spinal chord (cervical, thoracic and lumbar) Gasserian ganglia Dorsal root ganglia Dorsal and ventral spinal nerve roots Proximal sciatic nerve Peroneal (fibular) & sural nerves Tibial nerve	
3.6 Further remarks		
	4. RESULTS AND DISCUSSION	
4.1 Body weight	At 50 mg/kg, male bodyweight was reduced at 7 and 14 days post dosing by about 10 and 6% respectively, compared with controls. Female bodyweight was unaffected. Table A6.9.1-1.	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (1st study)**	
4.2 **Clinical signs of toxicity**	Mortality Six treatment-related deaths (5 males and 1 female) occurred at the highest dose level, 50 mg/kg. Five of these rats died within two days of dosing. The sixth, a male, was found dead five days after dosing. Clinical signs Clinical signs were seen at the highest dose level from the time of dosing for up to six days (Details are given in Tables A6.9.1-2 and -3)	
4.3 **Clinical chemistry**	Not applicable	
4.4 **Pathology**	No treatment related changes	
4.5 **Histopathology**	No treatment related changes	
4.6 **Other**	FOB examinations (see Tables A6.9.1-4 and -5) Findings of FOB examinations performed 7 hours and 7 days after single oral treatment are summarised in Tables A6.9.1.4 and -5, respectively. There were no treatment-related changes after a 14-day recovery period. Motor activity examinations (see Table A6.9.1-6) Results of motor activity examinations performed 8 hours after single oral treatment are summarised in Table A6.9.1.6. There were no treatment-related changes after a 7-day or 14-day recovery period.	
	5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1 **Materials and methods**	Groups of 15 male and 15 female Harlan Sprague Dawley rats were given a single dose, by gavage, of either 0 (control), 0.5, 5.0 or 50.0 mg/kg bw of Fipronil in corn oil, at a dose volume of 10 ml/kg bw. Control animals were given the vehicle alone. Dosing was staggered over 4 days and the study terminated 16 days post-dosing. Homogeneity of the Fipronil / vehicle mix and its stability were established prior to the start of dosing. Achieved concentrations in the dose preparations were verified at all dose levels prior to administration. Animals were examined twice daily for mortality and clinical signs of toxicity. Individual bodyweights were recorded the day before dosing, 7 hours after dosing, at weekly intervals thereafter (Days 7 and 14) and on the day prior to termination (Days 15 to 18). Neurobehavioural effects were evaluated in a functional observations battery and motor activity tests were performed prior to the start of dosing and at weekly intervals in conjunction with the bodyweight measurements. The rats were 4 weeks old on arrival and were acclimatised for approx. 3 weeks. On the day of treatment the animals weighed between 221.8 to 291.0 g (males) and 144.5 to 186.3 g (females). Rats were housed individually. 15 days after dosing, 10 animals/sex/group were anaesthetised, perfused with fixative (neutral buffered formalin), examined macroscopically. Brain, spinal cord, trigeminal nerve, Gasserian ganglia, dorsal root ganglia and associated nerve roots and sciatic, peroneal, sural and tibial peripheral nerves were preserved. The remaining five animals/sex/group were discarded. Tissues from six male and six female control and high dose (50.0 mg/kg bw) animals were evaluated microscopically for neuropathological changes.	X X
5.2 **Results and discussion**	Analysis of dose preparations	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (1st study)**	
	The mean achieved concentration of Fipronil in the dosing preparations were within the range of 93.0 to104.4% of the nominal dose, i.e. within the acceptable range. <u>Mortality and clinical signs</u> Six treatment-related deaths (5 males and 1 female) occurred the highest dose level, 50 mg/kg bw. Five of these rats died within two days of dosing. The sixth, a male, was found dead five days after dosing. Although the five that died within two days all exhibited a diffuse brain haemorrhage, this could have reflected agonal changes related to hypoxia rather than the cause of death. Treatment-related clinical signs were restricted to the highest dose level. Clonic/tonic convulsions occurred in 4 males and 1 female that died within 1 day of dosing. A further five males and one female surviving to termination had convulsions within the first day post-dosing but these did not recur. Other treatment-related clinical signs seen in several animals were indicative of cachexia. They included emaciation, dehydration, unkempt appearance, urine staining, cold extremities and/or pallor. Generally they occurred within two days of dosing and persisted for 1 to 6 days. <u>Bodyweight</u> At 50 mg/kg bw, male bodyweight was reduced by about 10% and 6% on days 7 and 14, respectively, compared with controls. Female bodyweight was unaffected. **<u>Functional Observation Battery (FOB)</u>** Treatment-related findings were generally restricted to the 7-hour post-dosing time point and predominantly at the highest dose level, 50 mg/kg bw/day, where responses tended to be more marked in males than in females. All findings had resolved by Day 14. 7-hour examination time point At 50 mg/kg bw, homecage treatment-related observations at the 7-hour post dosing time point included drooping or half-shut eyelids in males. During open field assessments, a mixture of responses was seen. Treatment-related stimulation of motor systems was evidenced as convulsions, tremors, head bobbing, myoclonic movements and decreased hind leg splay. In contrast, activity in the open field was depressed as indicated by decreased arousal and rearing activity and decreases in several reflexes (including approach response, tail pinch response, air-righting reflex). Reduced muscle tone, altered gait, decreased pupil size and reduced body temperature was also observed. At 5.0 mg/kg bw, the only treatment-related finding was a reduction in hind leg splay in both males and females. The statistically significant reductions in rearing in females from the intermediate and low dose levels were considered not to be related to treatment.	X

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (1st study)**	
	Day 7 post-dosing examination time point At the high-dose of 50 mg/kg bw, activity in males was apparently stimulated 7 days post dosing. This was indicated by changes in arousal (inactive and not alert to inactive but alert, alternating behaviour and hyperactive), open field rearing and exaggerated reflex responses to sound and tactile stimulation. The biological significance of this apparent stimulation is unclear because of the lack of effects on motor activity at this time point, the reversibility of the findings (no effect occurred at Day 14) and the lack of neuropathological lesions. Day 14 post-dosing examination time point None of the functional observations was considered to be treatment-related. **Motor activity** Treatment-related effects were restricted to a decrease in motor activity 8 hours post dosing in both sexes given 50 mg/kg bw. Findings at 7 and 14 days post-dosing were considered not to be related to treatment. Necroscopy and histopathology No treatment-related macroscopic or microscopic changes were found.	
5.3 Conclusion	A single oral administration of 50 mg/kg bw of Fipronil to rats caused mortality and a variety of changes to nervous system function including clinical signs, functional observations and motor activity responses principally at 7 hours post dosing. There were no associated histopathological changes. At 5.0 mg/kg bw, one slight functional effect on the nervous system was seen at 7 hours post dosing. The No Observed Effect Level (NOEL) was 0.5 mg/kg for both neurotoxicity and general toxicity.	
5.3.1 LO(A)EL	Neurotoxicity: 5 mg/kg bw, Based on reduction of hind-leg splay in both sexes 7-h after dosing. Reversible within 7 days after treatment. General toxicity: 50 mg/kg bw, based on mortality, reductions in body weight in males and unspecific clinical signs of toxicity in both sexes	
5.3.2 NO(A)EL	Neurotoxicity: 0.5 mg/kg bw General toxicity: 0.5 mg/kg bw	
5.3.3 Reliability	1	
5.3.4 Deficiencies	None	

Table A6.9.1-1 Group mean bodyweights

Time post-dosing	Dose level (mg/kg bw)							
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
	Males				Females			
Prior to dosing	211.12	207.14	208.96	212.09	147.77	145.16	148.28	147.14
7 hours	250.07	248.65	249.73	246.81	165.31	165.52	166.65	164.18
7 days	289.59	286.92	285.95	259.58**	186.19	187.66	190.60	181.13
14 days	321.63	318.44	316.88	302.51**	203.77	203.03	210.04	201.91

Statistical evaluation: ** p≤0.01

Table A6.9.1-2 Individual incidence of convulsions at 50 mg/kg bw

Animal number	Sex	Day found dead#	During clinical observations	During FOBs (7 hours post dosing)
1004	M	-	-	Clonic
1016	M	-	Tonic and clonic (Day 2)	-
1019	M	-	Clonic (Day 2)	-
1022	M	-	Tonic and clonic (Day 2)	-
1029	M	-	-	Clonic
1035	M	Day 2	Clonic (Day 1)	Clonic
1047	M	Day 3	-	Clonic
1062	M	-	-	Clonic
1124	F	Day 2	Clonic (Day 2)	-
1127	F	-	-	Clonic

all others survived to termination
FOB – Functional observation battery

Table A6.9.1-3 Group incidence of clinical signs

Finding	Dose level (mg/kg)							
	Males				Females			
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
Convulsions								
- clonic	0	0	0	4	0	0	0	1
- tonic	0	0	0	2	0	0	0	0
Emaciation	0	0	0	1	0	0	0	0
Dehydration	0	0	0	6	0	0	0	6
Unkempt	0	0	0	1	0	0	0	0
Urine staining	0	0	0	4	0	0	0	6
Cold extremities	0	0	0	2	0	0	0	0
Pallor of limbs	0	0	0	1	0	0	0	0
Dehydrated	0	0	0	6	0	0	0	6
Perioral encrustation	0	0	0	13	0	0	0	9

Table A6.9.1-4 Group incidence of functional observations 7 hours post dosing

Observations		Dose level (mg/kg bw)							
		0	0.5	5.0	50.0	0	0.5	5.0	50.0
		Males				Females			
Eyelids	- slight droop	0	1	0	4	0	0	0	0
	- half shut	0	0	0	1	0	0	0	0
Gait	- normal	15	15	15	5**	11	14	14	7
	- splayed	0	0	0	8	0	0	0	4
	- hypotonic	0	0	0	2	0	0	0	0
	- walking on toes	0	0	0	0	4	1	1	4
Clonic convulsion		0	0	0	4	0	0	0	1
Fine tremors	- none	15	15	15	9*	15	15	15	9*
	- whole body	0	0	0	1	0	0	0	4
	- limbs	0	0	0	5	0	0	0	1
	- head	0	0	0	0	0	0	0	1
Coarse tremors	- none	15	15	15	10*	15	15	15	14
	- whole body	0	0	0	5	0	0	0	0
	- limbs	0	0	0	0	0	0	0	1
Unusual behaviour									
	- none	15	15	15	13	15	15	15	12
	- head bobbing/weaving	0	0	0	1	0	0	0	1
	- myoclonic movements	0	0	0	1	0	0	0	2
Arousal behaviour	- alternating	7	13	10	4	8	13	13	3
	- hyperactive	1	0	0	0	7	1	2	0
	- inactive/alert	7	2	5	11	0	1	0	12
Palpebral closure									
	- wide open	14	15	14	11	15	15	15	12
	- slightly drooping	1	0	1	4	0	0	0	3
Rears (events)		3.67	6.53	3.93	0.20*	18.00	9.07**	10.53**	0.60**
Approach response	- noticeable	15	15	13	9*	15	15	15	15
	- none	0	0	2	6	0	0	0	0
Tale pinch response	- noticeable	13	14	14	9	14	15	15	12
	- none	1	1	1	6	0	0	0	3
Pupil size	- normal	14	15	15	6**	10	11	13	7
	- decreased	1	0	0	9	5	4	2	8
Muscle tone	- normal	15	15	15	10*	14	15	14	5**
	- decreased	0	0	0	5	1	0	1	10
Rectal temperature (°C)		37.95	38.08	38.23**	35.21**	38.75	38.48	38.45	35.79**
Air righting	- feet co-ordinated	14	15	15	7*	15	15	15	15
	- feet uncoordinated	1	0	0	1	0	0	0	0
	- back	0	0	0	4	0	0	0	0
	- side	0	0	0	3	0	0	0	0
Hind leg splay (cm)		7.92	7.18	7.00*	5.72**	7.54	7.64	6.55*	5.34**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$

Table A6.9.1-5 Group incidences of functional observations 7 days post dosing

Observations	Dose level (mg/kg bw)							
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
	Males				Females			
Arousal:								
– alternating behaviour	5	5	9	7	9	10	13	10
– hyperactive	1	0	0	3	6	2	2	3
– inactive/alert	9	10	6	0	0	3	0	1
Rears (events)	3.53	3.13	6.60	10.20**	15.80	11.33	13.80	16.36
Startle response:								
– noticeable	15	~~5~~ 15	15	7	15	15	15	13
– exaggerated	0	0	0	3	0	0	0	1
Tale pinch response:								
– noticeable	14	12	14	8	15	14	15	13
– none	1	2	1	0	0	0	0	0
– exaggerated	0	1	0	2	0	1	0	1

Statistical evaluation: ** = $p \leq 0.01$

Table A6.9.1-6 Group mean motor activity 8 hours post dosing (cumulative session counts)

Time post dosing	Dose level (mg/kg bw)							
	0	0.5	5.0	50.0	0	0.5	5.0	50.0
	Males				Females			
Pre-treatment	502.9	623.6	623.2	630.7	822.3	833.1	841.7	736.5
8 hours	557.4	613.9	531.2	56.0**	981.5	945.3	1008.5	68.9**
7 days	584.8	752.6*	836.1*	709.2	1021.1	822.3	964.5	993.0
14 days	623.4	798.5	803.4	581.1	909.0	916.7	754.7	932.3

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Individual bodyweights were recorded the day before dosing,* ~~*7 hours after dosing,*~~ and *at weekly intervals thereafter* *Tissues from six* ~~*male and six female control and high dose (50.0 mg/kg bw) animals*~~ animals in the control and high dose groups 5.2 Results and discussion: *Clonic/tonic convulsions occurred in 4 males and 1 female* ~~*that died.*~~ Three of these animals were found dead *within 1 day* after convulsions ~~*of dosing*~~. *A further five-males and one female surviving to termination had convulsions within the* ~~*first day*~~ 7-hour *post-dosing but these did not recur.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
	1. REFERENCE	**Official use only**
1.1 Reference	**A6.9/02** XXXX Fipronil: Neurotoxicity to rats by acute oral administration (including a time to peak effect study). 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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	Yes EPA 81-8	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	X
3.1.1 Lot/Batch number	TAK 1747	
3.1.2 Specification	As given in Section 2	X
3.1.2.1 Description	White powder	
3.1.2.2 Purity	97.9%	
3.1.2.3 Stability	Stable	X
3.2 Reference Substance (positive control)	None included in this study.	
3.3 Test Animals		
3.3.1 Species	Rat	
3.3.2 Strain	Sprague-Dawley	X
3.3.3 Source	XXXX	
3.3.4 Sex	Male and female	
3.3.5 Rearing conditions	Normal animal husbandry	
3.3.6 Age/weight at study initiation	Age on arrival: males: 6 wk, females: 5 wk Acclimatisation period: at least 12 days Body weight range on treatment day: males 248–329 g; females 181–229 g	
3.3.7 Number of animals per group	10/group/sex	
3.3.8 Control animals	Yes	
3.4 Administration	Oral	
3.4.1 Exposure	Single dose by gavage	
3.4.2 Dose levels	0, 2.5, 7.5, or 25 mg/kg bw	
3.4.3 Vehicle	Corn oil	
3.4.4 Concentration in vehicle	0, 0.25, 0.75 or 2.5 mg/ml	
3.4.5 Total volume applied	10 ml/kg bw	
3.4.6 Postexposure period	14 days	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
3.4.7 Anticholinergic substances used	Not applicable (not an OP)	
3.4.8 Controls	Vehicle	
3.5 Examinations		
3.5.1 Body weight	Yes One week prior to dosing, on the day to dosing, 7 days after dosing. Food consumption was monitored at weekly intervals, starting one week prior to dosing	X
3.5.2 Signs of toxicity	Yes <u>Mortality and overt clinical signs:</u> twice daily on working days <u>Behavioural changes, reaction to treatment or ill health:</u> At least once daily, 7 days/week <u>Functional observational battery (FOB):</u> prior to dosing; time of peak effect (7 h), 7 days and 14 days post-dosing Home cage observations Observations in the hand Observations in the arena Manipulations: Approach, touch, startle, tail-pinch, and pupil responses; righting reflex, grip strength (fore and hindlimb), landing foot splay, body temperature and body weight. <u>Motor activity evaluations</u> Locomotor activity automatically measured prior to dosing; at time of peak effect (7 h), 7 days and 14 days post-dosing.	
3.5.3 Observation schedule	See above	
3.5.4 Clinical chemistry	No	
3.5.5 Pathology	Yes All animals 14 days after treatment (Day 15): Pentobarbital anaesthesia, In-situ perfusion fixation with heparinised 0.7% sodium nitrite followed by 1.5% glutaraldehyde / 4% paraform-aldehyde solution and tissue samples taken Brain: Fixed brain weight, anatomical measurement of width and length	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
3.5.6 Histopathology	Yes Tissues were processed and evaluated for 5 rats/sex for the high-dose and control groups. Tissues: Forebrain (three levels), Cerebellum and pons Midbrain Medulla oblongata Spinal chord (cervical C3-C6 and lumbar L1-L4) Gasserian (trigeminal) ganglia Dorsal root ganglia, and dorsal and ventral root fibres (one cervical and one lumbal level). The dorsal and ventrai root fibres and dorsai root ganglia examined in each case was one from any of C3-C6 and one from any of L1-L4. Sciatic nerve (sciatic notch and mid-thigh) Sural nerve (at and just distal to knee) Tibial nerve (at and just distal to knee)	
3.6 Further remarks	**4. RESULTS AND DISCUSSION**	
4.1 Body weight	(See Table A6.9.2-1) Bodyweight gain of both sexes given 25 mg/kg bw and of females dosed with 7.5 mg/kg bw was significantly reduced during the first week after treatment, but was unaffected during Week 2.	
4.2 Clinical signs of toxicity	Mortality and clinical signs (see Table A6.9.2-4) There were no deaths. Treatment-related clinical signs were restricted to the 25 mg/kg bw group. They consisted of poor grooming (staining of the fur of the head/muzzle/nasal regions and soiled/stained anogenital region) the day after treatment (Day 2) but disappeared largely within a few days. FOB examinations (Table A6.9.2-5) At 25 mg/kg bw stationary positioning was observed for the tail pinch response in some males and females (also noted in two males given 2.5 mg/kg bw) and an increased incidence of vocalisation of males. In addition, two animals (one male and one female) showed unusual behaviour. The male exhibited chewing in the arena with his chin resting on the floor and rubbing this on the floor. The female had ear twitches, became slightly awkward to handle and apparently limpness. In the arena it adopted a frozen posture with the lower jaw touching the floor. Forelimb grip strength was increased in males at 25 mg/kg bw whilst hind limb splay was reduced in both sexes. Mean body temperature was also significantly reduced at 25 mg/kg bw. The incidence of vocalisation, a non-specific sign, was slightly higher in males at this high dose level at all time points. This was the only finding at the 7 and 14 days observation times. At 7.5 mg/kg bw, the only finding was reduced hind limb splay at 7 hours post dosing. Motor activity examinations (see Table A6.9.2-6) At 25 mg/kg bw, locomotor activity was reduced in both sexes at 7 hours post dosing and specifically during the first 10 minutes of testing. It was not affected at Days 7 and 14.	
4.3 Clinical chemistry	Not applicable	
4.4 Pathology	No treatment related changes	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
4.5 Histopathology **4.6 Other**	No treatment related changes Food consumption and food conversion (see Tables A6.9.2-2 and -3) At 25 mg/kg bw, male food consumption was significantly reduced during Weeks 1 and 2 post dosing although some recovery was apparent during the second week. Consumption by females given 7.5 or 25 mg/kg bw was reduced only during Week 1. At 25 mg/kg bw, the efficiency of food utilisation was impaired in both sexes (slightly in males and markedly in females) during Week 1. It was also affected over the same period in females dosed with 7.5 mg/kg bw.	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** In this acute neurotoxicity study, Fipronil was administered orally, by gavage, to groups of 10 male and 10 female Sprague-Dawley rats in order to assess Fipronil's potential acute neurotoxicity and the time to peak effect. The rats were 6 weeks old (males) or 5 weeks old (females) on arrival and were acclimatised for approx. 2 weeks. They were housed individually. On the day of treatment they weighed between 248 to 329 g (males) and 181 to 229 g (females). Fipronil suspended in corn oil was given at single dose levels of either 0 (control), 2.5, 7.5 or 25 mg/kg bw at a dose volume of 10 ml/kg bw. Control animals were given the vehicle only. The study was terminated after a 14-day observation period. Prior to the start of dosing, trial mixtures were analysed to confirm the stability of Fipronil in the vehicle. Samples of the dose preparations were also taken on one day of dosing to verify the achieved concentration of test material. Animals were examined daily for mortality and clinical signs of toxicity. Individual bodyweights were recorded prior to treatment (Week -1), on the day of treatment (Day 0) and weekly thereafter (Days 7 and 14) until termination. They were also noted at the time of each functional observations battery. Individual food consumption was recorded weekly commencing Week -1. Effects on neurological function were assessed in functional observations batteries and motor activity tests conducted prior to dosing and at 7 hours, 7 and 14 days post dosing. At the end of the 14-day post-treatment observation period, animals were anaesthetised, perfused with fixative (sodium nitrite followed by 15% glutaraldehyde: 4% paraformaldehyde), examined macro-scopically and anatomical measurements were taken of the brain. Neural tissues were then preserved (brain, spinal cord, Gasserian and dorsal ganglia, dorsal and ventral root fibres and sciatic, sural and tibial peripheral nerves). Tissues from five males and females from each of the control and highest dose groups (25 mg/kg bw) were examined microscopically.	
5.2 Results and discussion	Analysis of dose preparations The achieved concentrations of Fipronil in the dose preparations were in the range of 94–96.7% of intended nominal dose levels and thus considered to be acceptable.	

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
	Bodyweight (Table A6.9.2-1) Bodyweight gain of both sexes given 25 mg/kg bw and of females dosed with 7.5 mg/kg bw was significantly reduced during the first week after treatment, but was unaffected during Week 2. Food consumption (Table A6.9.2-2) At 25 mg/kg bw, male food consumption was significantly reduced during Weeks 1 and 2 post dosing although some recovery was apparent during the second week. Consumption by females given 7.5 or 25 mg/kg bw was reduced only during Week 1. Food conversion ratio (Table A6.9.2-3) At 25 mg/kg bw, the efficiency of food utilisation was impaired in both sexes (slightly in males and markedly in females) during Week 1. It was also affected over the same period in females dosed with 7.5 mg/kg bw. Mortality and clinical signs (Table A6.9.2-4) There were no deaths. Treatment-related clinical signs were restricted to the 25 mg/kg bw group. They consisted of poor grooming (staining of the fur of the head/muzzle/nasal regions and soiled/stained anogenital region) the day after treatment (Day 2) but disappeared largely within a few days. **Functional Observation Battery (FOB)** At 25 mg/kg bw, changes that were considered treatment-related comprised stationary positioning for the tail pinch response in some males and females (also noted in two males given 2.5 mg/kg bw) and an increased incidence of vocalisation of males. In addition, two animals (one male and one female) showed unusual behaviour. The male exhibited chewing in the arena with his chin resting on the floor and rubbing this on the floor. The female had ear twitches, became slightly awkward to handle and apparently limpness. In the arena it adopted a frozen posture with the lower jaw touching the floor. Forelimb grip strength was increased in males at 25 mg/kg bw whilst hind limb splay was reduced in both sexes. Mean body temperature was also significantly reduced at 25 mg/kg bw. The incidence of vocalisation, a non-specific sign, was slightly higher in males at this high dose level at all time points. This was the only finding at the 7 and 14 days observation times. At 7.5 mg/kg bw, the only finding was reduced hind limb splay at 7 hours post dosing. 7-hour examination time point (see Table A6.9.2-5) At 25 mg/kg bw, treatment-related changes comprised stationary positioning for the tail pinch response in some males and females (also noted in two males given 2.5 mg/kg bw) and an increased incidence of vocalisation of males. In addition, two animals (one male and one female) showed unusual behaviour. The male exhibited chewing in the arena with his chin resting on the floor and rubbing this on the floor. The female had ear twitches, became slightly awkward to handle and apparently limpness. In the arena it adopted a frozen posture with the lower jaw touching the floor.	X

Section A6.9 **Annex Point IIIA, VI.1**	**Acute neurotoxicity in rats (2nd study)**	
	Forelimb grip strength was increased in males at 25 mg/kg bw whilst hind limb splay was reduced in both sexes. Mean body temperature was also significantly reduced at 25 mg/kg bw. At 7.5 mg/kg bw, the only finding was reduced hind limb splay. <u>Days 7 and 14 post-dosing examination</u> (see Table A6.9.2-5) The incidence of vocalisation, a non-specific sign, remained to be slightly higher in males at 25 mg/kg bw at both time points. There were no treatment-related effects at 7.5 or 2.5 mg/kg bw. **<u>Motor activity</u>** (see Table A6.9.2-6) At 25 mg/kg bw, locomotor activity was reduced in both sexes at 7 hours post dosing and specifically during the first 10 minutes of testing. It was not affected at Days 7 and 14. <u>Necroscopy and histopathology</u> There were no treatment-related macroscopic or microscopic changes and no effects on brain weight or morphometric (anatomical) brain measurements.	
5.3 Conclusion	A single oral gavage administration of 25 mg/kg bw of Fipronil to rats reduced bodyweight gain and food consumption and impaired food utilisation during the week following treatment. Behavioural changes were also seen 7 hours post dosing with one male and one female exhibiting unusual behaviour/posture. Reduced body temperature, an increased incidence of forelimb grip strength in males, decrease in landing footsplay and reduced locomotor activity also occurred at this time. There were no neuropathological changes. At 7.5 mg/kg bw of Fipronil, female bodyweight gain, food consumption and food conversion efficiency were reduced during the first week post dosing. Behavioural findings were limited to a decreased hind leg splay in males at 7 hours post dosing. The time to peak effect was 7 hours post dosing. The No Observed Effect level (NOEL) for neurobehavioural and general toxicity was 2.5 mg/kg bw.	
5.3.1 LO(A)EL	<u>Neurotoxicity:</u> 7.5 mg/kg bw, based on decreased hindleg splay in males at 7 hours post dosing <u>General toxicity:</u> 7.5 mg/kg bw, based on reduced female body weight gain, food consumption and food conversion during the first week after dosing.	
5.3.2 NO(A)EL	<u>Neurotoxicity:</u> 2.5 mg/kg bw <u>General toxicity:</u> 2.5 mg/kg bw	
5.3.3 Reliability	1	
5.3.4 Deficiencies	None	

Table A6.9.2-1 Group mean bodyweights and bodyweight gains (g)

Weeks	Dose level (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
Bodyweight								
-1	242	242	239	238	182	183	182	180
0 (dosing)	293	288	288	281	211	208	206	208
1	325	316	315	298	227	223	213	212
2	360	347	341	330	244	242	225	231
Bodyweight gain								
0 to 1	32	28	27	17**	16	15	7**	4**
1 to 2	35	31	26	33	17	18	12	19

Statistical evaluation: ** p≤0.01

Table A6.9.2-2 Group mean food consumption (g/rat/week)

Week	Dosage (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
-1	227	226	221	212	153	146	140	153
1	222 (100)	211 (95)	207 (93)	165** (74)	165 (100)	152 (92)	138** (84)	125** (76)
2	216 (100)	210 (97)	201* (93)	195** (90)	155 (100)	151 (97)	139 (90)	154 (99)

% of control in parenthesis; Statistical evaluation: * p≤0.05; ** p≤0.01

Table A6.9.2-3 Group mean food conversion ratios (food consumption/body weight gain)

Week	Dose level (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
1	7.0	7.4	7.7	9.7	10.2	9.9	20.2	30.8
2	6.2	6.8	7.8	6.0	9.1	8.3	11.3	8.0
Overall mean	6.6	7.1	7.7	7.3	9.6	9.0	14.5	12.0

Table A6.9.2-4 Group incidence of clinical signs

	Dose level (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
Day 2								
Staining on head/face	0	0	0	5	0	0	0	6
Soiled anogenital area	0	0	0	3	0	0	0	0
Soft faeces	0	0	0	1	0	0	0	0
Matted fur	0	0	0	1	0	0	0	0
Post Day 2								
Staining on head/face	3	6	4	4	1	1	3	0

Table A6.9.2-5 Functional observations 7 hours post dosing (except where indicated)

Observations	Dose level (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
Number of animals tested	10	10	10	10	10	10	10	10
Stationary position for tail pinch response	0	2	0	3	0	0	0	2
Vocalisation								
Pre-treatment	2	2	2	3	1	1	0	2
7 hours post dosing	1	1	2	4	2	0	1	2
7 days post dosing	1	2	1	3	2	2	1	1
14 days post dosing	0	1	3	3*	0	1	1	0
Fore limb grip strength (kg)	0.91	0.98	0.97	1.11**	0.90	0.90	0.94	0.97
Hind leg splay (cm)	8.7	7.8	6.7**	6.3**	7.1	7.1	6.1	5.6*
Body temperature (°C)	37.9	38.0	37.9	37.6*	38.2	38.1	38.2	37.6**

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$

X

Table A6.9.2-6 Locomotor activity (mean large movements/sec)

Time post-dosing	Dose level (mg/kg bw)							
	0	2.5	7.5	25	0	2.5	7.5	25
	Males				Females			
1 hour	304	294	314	212	303	320	296	176
First 10 minutes	218	205	200	107**	195	203	184	113**

Statistical evaluation: ** $p \leq 0.01$

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.3.2 Strain: ~~*Sprague-Dawley*~~ Crl: CD BR 3.5.1 Body weight: *Yes One week prior to dosing, on the day to dosing~~, 7 days after dosing~~* and weekly thereafter (days 7 and 14)
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *7-hour examination time point:* There was a slight increase in the incidence in nasal staining among females treated at 25 mg/kg. No related to treatment: Stationary position at 2.5 mg/kg 7-hour post dosing (this finding was not observed at 7.5 mg/kg), stretching movements among males at 7-hour post treatment (increased frequency among controls), the change in walking on toes on day 7 for animals treated at 25 mg/kg (animals showed this change in the pre-dose period), the touch response on day 7 for animals treated at 7.5 and 25 mg/kg ("deficit" appeared to be among the controls) and the decreased frequency of grooming among females treated at 7.5 or 25 mg/kg on days 7 and 14 (no other changes in behaviour were observed).
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.9 **Annex Point IIIA6.1**	**Subchronic neurotoxicity**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.9/03** XXXX M&B 46030: Ninety-day dietary neurotoxicity study in Sprague Dawley rats. 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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes US EPA 82-5(b)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	78/GC/90	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	White powder	
3.1.2.2	Purity	96.7%	
3.1.2.3	Stability	Stable	**X**
3.2	**Reference Substance (positive control)**	None included in this study. Observers were trained in regular proficiency test sessions (listed tests from 1988-1993) with a variety of positive control compounds including Chlorpromazine (leg splay, decreased startle response), amphetamine, (stereotypy, piloerection), acrylamide (limb weakness), and DDT (tremor).	
3.3	**Test Animals**		
3.3.1	Species	Rat	
3.3.2	Strain	Sprague-Dawley	
3.3.3	Source	Harlan Sprague Dawley Inc, Indianapolis, USA	
3.3.4	Sex	Male and female	
3.3.5	Rearing conditions	Normal animal husbandry	
3.3.6	Age/weight at study initiation	35 days Body weight range: males 251.2 to 279.9 g; females 162.6 to 192.3 g	**X**
3.3.7	Number of animals per group	15/group/sex	
3.3.8	Control animals	Yes	
3.4	**Administration**	Oral diet	
3.4.1	Exposure	13 weeks	
3.4.2	Dose levels	0 (control), 0.5, 5.0 or 150 ppm in the diet (mg fipronil/kg diet) corresponding to intakes males: 0.03, 0.30, 8.89 mg/kg bw/d females: 0.04, 0.35, 10.78 mg/kg bw/d	**X**
3.4.3	Vehicle	Diet	

Section A6.9 **Annex Point IIIA6.1**		**Subchronic neurotoxicity**	
3.4.4	Concentration in vehicle	0 (control), 0.5, 5.0 or 150 ppm in the diet	
3.4.5	Total volume applied	Not applicable	
3.4.6	Postexposure period	None	
3.4.7	Anticholinergic substances used	None	
3.4.8	Controls	Plain diet	
3.5	**Examinations**		
3.5.1	Body weight	Yes: Weekly until termination	
3.5.2	Signs of toxicity	Yes <u>Mortality and overt clinical signs:</u> twice daily, 7 days/week <u>Functional observational battery (FOB) and motor activity evaluations:</u> All animals, 1 week prior to dosing and during treatment weeks 4, 9, and 13	
3.5.3	Observation schedule	See above	
3.5.4	Clinical chemistry	No	
3.5.5	Pathology	Yes 10 animals/group/sex (150 ppm females: 11 animals) After perfusion fixation, examination of pleural cavity including heart, lungs, and thymic area, and of the abdominal cavity with liver, kidneys, spleen and adrenals. - Fixed brain weight	
3.5.6	Histopathology	Yes 6 animals/sex for control and high-dose group Brain (cerebral cortex, cerebellar cortex, medulla/pons), spinal chord (cervical, thoracic, lumbar), dorsal root ganglia, dorsal and ventral nerve roots, Gasserian ganglia, sciatic nerve, tibial nerve, sural and peroneal nerves	**x**
3.6	**Further remarks**		**x**
		4. RESULTS AND DISCUSSION	
4.1	**Body weight**	At 150 ppm, bodyweight gain was reduced in both sexes in Week 1 (by approximately 38 and 30% of control values in males and females, respectively). Male weight gain was also reduced in Week 2 (approximately 83% of control values).	
4.2	**Clinical signs of toxicity**	One female given 0.5 ppm was found dead on Day 16 without having shown any clinical signs. Necropsy revealed severe bilateral renal pelvic dilatation other related observations. Therefore this isolated mortality was considered incidental. No treatment-related clinical signs were seen.	
4.3	**Clinical chemistry**	Not applicable	
4.4	**Pathology**	No treatment related changes	
4.5	**Histopathology**	No treatment related changes	
4.6	**Other**	None	**x**
		5. APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Groups of 15 male and 15 female Sprague Dawley rats were given Fipronil continuously for 13 weeks at dietary concentrations of either 0 (control), 0.5, 5.0 or 150 ppm in order to determine and evaluate its potential neurobehavioural and neuroanatomic effects.. Control animals were given untreated diet. The study was terminated during	

Section A6.9 **Annex Point IIIA6.1**		**Subchronic neurotoxicity**	
		Week 14. The rats were 35 days old on arrival and acclimatised for approximately 3 weeks. At the start of treatment they weighed between 251.2 to 279.9 g (males) and 162.6 to 192.3 g (females). They were housed individually throughout. Stability of the test material in the diet over 14 and 21 days and homogeneity of mixing was checked at 0.5 and 300 ppm. Fresh diet was prepared weekly. Samples of each dietary concentration from Weeks 8 and 13 were analysed for achieved concentration of Fipronil. Animals were examined twice daily for mortality and clinical signs of toxicity. Individual bodyweights and food consumption were recorded weekly until termination. Neurological behaviour was assessed in functional observations batteries and motor activity tests conducted prior to the start of treatment and during Weeks 4, 9 and 13 of treatment. At the end of the treatment period (Week 14), 10 animals/sex/group (11 females at the highest dose level) were anaesthetised, perfused with fixative (10% neutral buffered formalin), examined macroscopically and tissues preserved (brain, spinal cord and sciatic, peroneal, sural and tibial peripheral nerves). The remaining five animals/sex/group were discarded. A histopathological examination for neuropathological changes was performed on tissues from six male and six female rats of the control and high dose groups (150 ppm).	
5.2	**Results and discussion**	Analysis of dose preparations Analysis of the stability samples at 0.5 and 300 ppm of Fipronil gave mean measured concentrations between 90.1 and 103.1% of nominal. Homogeneity of mixing was confirmed. Analysis of samples from all dietary dose levels for achieved concentrations gave mean achieved values within the range 90.2 to 104.8% of nominal i.e. within the acceptable range. Mortality and clinical signs One female given 0.5 ppm was found dead on Day 16 without having shown any clinical signs. Necropsy revealed severe bilateral renal pelvic dilatation other related observations. Therefore this isolated mortality was considered incidental. No treatment-related clinical signs were seen. Test substance intake See Table A6.9.3-1 Bodyweight (see Table A6.9.3-2) At 150 ppm, bodyweight gain was reduced in both sexes in Week 1 (by approximately 38 and 30% of control values in males and females, respectively). Male weight gain was also reduced in Week 2 (approximately 83% of control values). Food consumption (see Table A6.9.3-3) At 150 ppm, food consumption was significantly reduced in both males and females in Week 1 (approximately 77 and 76% of control values, respectively), but was unaffected thereafter. Functional observations There were no treatment-related findings in the functional observation batteries at any time point. Motor activity Motor activity was unaffected by treatment.	**x** **x**

Section A6.9 **Annex Point IIIA6.1**	**Subchronic neurotoxicity**	
5.3 Conclusion	Histopathology There were no treatment-related macroscopic or microscopic changes. Continuous dietary administration of 150 ppm of Fipronil to rats for 90 days reduced bodyweight gain and food consumption during Weeks 1 or 2. There was no evidence of any neurological effect. The NOAEL for neurotoxicity was 150 ppm (corresponding to 8.9 mg/kg bw/d in males and 10.8 mg/kg bw/d in females). The NOAEL 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).	
5.3.1 LO(A)EL	General toxicity: 150 ppm (transient reduction of body weight gain and food consumption) Neurotoxicity: > 150 ppm (no evidence of neurotoxicity under the conditions of this study)	
5.3.2 NO(A)EL	General toxicity: 5 ppm (corresponding to 8.9 mg/kg bw/d in males and 10.8 mg/kg bw/d in females) Neurotoxicity: ≥ 150 ppm (corresponding to 8.9 mg/kg bw/d in males and 10.8 mg/kg bw/d in females)	**x**
5.3.3 Reliability	1	
5.3.4 Deficiencies	None	

Table A6.9.3-1 Group mean test substance intake (mg/kg bw/d)

	Dose level (ppm)					
	Males			**Females**		
	0.5	**5.0**	**150**	**0.5**	**5.0**	**150**
Weeks 1-13	0.03	0.30	8.89	0.04	0.35	10.78

Table A6.9.3-2 Group mean bodyweights

Week	**Dose level (ppm)**							
	Males				**Females**			
	0	**0.5**	**5.0**	**150**	**0**	**0.5**	**5.0**	**150**
Bodyweight (g)								
0	266.4	268.1	266.6	266.4	177.1	176.9	178.1	175.6
1	295.4	300.9	296.9	277.4**	191.3	193.1	192.5	179.9**
2	315.5	325.2*	319.2	307.3	203.2	204.1	204.8	197.7
5	353.5	367.8	364.0	359.4	229.1	229.2	228.9	222.2
13	421.8	434.4	435.6	442.2	263.8	261.9	262.9	262.5
Bodyweight gain (g)								
0-1	28.9	32.8*	30.4	11.0**	14.2	16.2	14.3	4.2**
0-2	49.1	57.1**	52.6	40.9**	26.0	27.2	26.7	22.1
0-5	87.0	99.6**	97.4*	93.0	52.0	51.6	50.8	46.6
0-13	155.4	166.3	169.1	175.8	86.6	84.4	84.8	86.9

Statistical evaluation: * $p \leq 0.05$; ** $p \leq 0.01$

Table A6.9.3-3 Group mean food consumption (g/rat/day)

Week	Dose level (ppm)							
	Males				Females			
	0	0.5	5.0	150	0	0.5	5.0	150
1	22.4	22.7	22.6	17.2**	16.5	16.8	16.1	12.5**
2	22.1	22.5	22.5	22.8	16.7	16.4	16.3	17.1
13	20.5	20.5	21.2	21.1	15.1	15.5	15.4	15.1

Statistical evaluation: ** $p \leq 0.01$

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.3.6 Age/weight at study initiation: ~~*35 days*~~ 8 weeks 3.4.2 Dose levels: The range of daily test substance intake for the males over 13 weeks was approximately 40-24 µg/kg/d for the 0.5 ppm group, 401-245 µg/kg/d for the 5.0 ppm group and 11696-7210 µg/kg/d for the 150 ppm group. The dose that females received was somewhat higher based upon their generally lower body weights. The low dose group received approximately 46-30 µg/kg/d, 436-294 µg/kg/d for the mid dose group and 13614-8626 µg/kg/d for the high dose group. 3.5.6 Histopathology: + thyroids and liver 3.6 Further remarks: food consumption: weekly
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.6 Other: ~~*None*~~ Food consumption : At 150 ppm, food consumption was significantly reduced in both males and females in Week 1 (approximately 77 and 76% of control values, respectively), but was unaffected thereafter.
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.2 Results and discussion: *Analysis of the stability samples at 0.5 and 300 ppm of Fipronil gave mean measured concentrations between 90.1 and 103.1%* and between 93.0 and 95.6% *of nominal* respectively. The diets were analyzed after preparation (day 0) , and following 7, 14, 21 days of storage. *Bodyweight:* At 5.0 ppm, males showed a significant increase in cumulative body weight gain for the week 0 to 5 interval. Significant increases in male cumulative weight gain were also evident in the 0.5 ppm dose group for each interval of comparison through week 5. Body weights were significantly increased for the males in the low dose group at weeks 2 and 3. For the subset of males that were evaluated with the FOB, the increases in body weight were even more prominent, and significant body weight increases were present in all three treated groups of males. The mean body weight of the low dose group was increased at weeks 4 and 9; the mid dose group was increased at weeks 4, 9 and 13. Body weights were also increased for the high dose group at weeks 9 and 13. The mid and high dose groups also had significantly increased final body weights just prior to sacrifice. 5.3.2 NO(A)EL: General toxicity: *5 ppm* ~~*(corresponding to 8.9 mg/kg bw/d in*~~

	~~*males and 10.8 mg/kg bw/d in females)*~~ (corresponding to 0.3 mg/kg bw/d in males and 0.4 mg/kg bw/d in females)
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies**

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**

		Official use only
	1. REFERENCE	
1.1 Reference	**A6.10/01** XXXX M+B 46,030: An investigation into the potential effects on thyroid function in male rats by studying thyroxine clearance. 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 to Annex 1	
	2. GUIDELINES AND QUALITY ASSURANCE	
2.1 Guideline study	No No guideline exists for this mechanistic investigation	
2.2 GLP	Yes	
2.3 Deviations	No	
	3. MATERIALS AND METHODS	
3.1 Test material	As given in Section 2	x
3.1.1 Lot/Batch number	PGS 963	
3.1.2 Specification	As given in Section 2	x
3.1.2.1 Description	Off-white powder	
3.1.2.2 Purity	95.4%	
3.1.2.3 Stability	Stable	x
3.2 Test Animals		
3.2.1 Species	Rat	
3.2.2 Strain	Sprague-Dawley (Crl: CD(SD))	
3.2.3 Source	XXXX	
3.2.4 Sex	Male, approx. 49 days old, weighing between 282–306 g at the start of dosing	x
3.2.5 Number of animals per group	6	
3.2.6 Control animals	Yes	
3.3 Administration/ Exposure	Oral	
3.3.1 Duration of treatment	a) 1 day b) 14 days	
3.3.2 Frequency of exposure	Once daily	
3.3.3 Postexposure period	Up to 34 hours after final dose (up to 30 hours after administration of radiolabelled thyroxine	
3.3.4 Oral		
3.3.4.1 Type	Oral gavage	
3.3.4.2 Dose	10 mg/kg bw	
3.3.4.3 Vehicle	0.5 % aqueous methylcellulose	
3.3.4.4 Concentration in vehicle	2 mg/ml	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**	
3.3.4.5 Total volume applied	5 ml/kg bw	
3.3.4.6 Controls	Negative control: Vehicle (0.5 % aqueous methylcellulose) Positive control: Phenobarbital (i.p. dose: 80 mg/kg bw/d, volume: 2 ml/kg bw)	
3.3.4.7 Administration of test materials	Subset groups were treated either once or daily for 14 days with the vehicle, with Fipronil or with Phenobarbital. On the last day of treatment, each group each rat received 1 mg NaI in 0.9% saline solution (0.5 ml) by intraperitoneal injection. This solution was administered on two occasions, i.e. 5 minutes and approx. 10 hours 5 minutes after the last test compound administration. Similarly, each rat received [^{125} I]thyroxine (T_4) in 0.9% saline solution (10 µCi/kg bw) using a constant dosage volume of 2 ml/kg bw by intravenous injection, 4 hours after last test compound administration.	
3.4 Examinations		
3.4.1 Clinical signs and mortality	Check for mortality and moribund animals: twice daily, 7 days/week Check for any signs of behavioural changes, reaction to treatment or ill health: at least once daily	
3.4.2 Bodyweight	The weight of each rat was recorded at the time of allocation of animals to groups, on the day of commencement of treatment, pre-terminally for single-dosed rats and once a week thereafter for repeatedly dosed rats. Bodyweights were recorded also on Day -1 and Day 13. These bodyweight data were used to calculate dosage volumes for administration of [^{125} I]thyroxine (T_4).	
3.4.3 Food consumption	Single-dose group rats: Day -2 to Day 3, cage-wise Repeated-dose group rats: Day -2 to Day 7, cage-wise; subsequently on Day 14 and at termination of the study	
3.4.4 Thyroxine clearance	Blood samples were obtained from the tail vein of all single-dosed animals of each group on Day 1, and from all repeatedly dosed animals of each group on Day 14. Blood was withdrawn 0.5, 1, 2, 4, 8, 12, 20, 24 and hours after administration of [^{125} I]thyroxine:. Aliquots were processed and examined by gamma counting for determination of total radioactivity, protein-bound activity and non-protein bound activity.	X
	4. RESULTS AND DISCUSSION	
4.1 Mortality	There were no deaths during the treatment period	
4.2 Clinical Signs	No findings considered attributable to treatment with Fipronil were noted. Collapsed posture and lethargy after dosing were noted for Phenobarbital treated animals. Shallow breathing was noted for Phenobarbital-treated animals on the first day of treatment only.	
4.3 Bodyweight	Group mean body weights of animals treated with Fipronil or with Phenobarbital were similar to those of the control animals over the treatment period.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**	
4.3 Thyroxine Clearance	(See Tables A6.10.1-1 and -2) Concentrations of thyroxine in whole-blood samples were calculated from the dpm values and the specific activity of [^{125} I] thyroxine (1200µCi/µg). From Figures 6.10.1-1 and 2 it is apparent that there was a biphasic distribution of thyroxine in whole-blood with time, indicative of an absorption phase followed by an excretion/metabolism phase. To determine whether any free ^{125} I had been released from the thyroxine peptide, whether through metabolic deiodination or through chemical dissociation, thereby contributing to levels of ^{125} I measured in whole-blood, aliquots of these samples of whole-blood were treated with trichloroacetic acid in order to precipitate out blood proteins. Following centrifugation o f the acidified solution, portions of the resulting supernatant were taken for gamma counting. For no group of animals was the amount of ^{125} I detected in supernatants of acidified blood regarded as being significantly above background levels. Since it is known that thyroxine is largely bound to blood proteins under normal physiological conditions, it is concluded that the [^{125} I] thyroxine used in the present study was stable in whole-blood, at least over the period that samples were analysed, and consequently that levels of radioactivity measured could be related directly to amounts of thyroxine.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**	
	The data for concentrations of thyroxine in whole-blood (summarised in Table A6.10.1-1) were subsequently used to generate pharmaco-kinetic parameters, including the terminal half-life, clearance and volume of distribution of thyroxine in whole-blood (see Table A6.10.1-2). Statistical analyses indicated significant increases in the terminal half-life and the volume of distribution (of 31 and 37% respectively) for control animals that had been maintained for 14 consecutive days, compared with those maintained for only 1 day. Given these age-related changes, statistical comparisons between Day 1 and Day 14 values for rats in the treated groups were not reported, since it would be unclear how much of the change was due to age and how much to length of time on treatment. There was no evidence for any significant action of Fipronil after 1 day of dosing but significant effects were noted after 14 days of dosing. These included a highly significant decrease in the terminal half-life (45%) and increases in the clearance (161%) and volume of distribution (37%). (See Table A6.10.1-2) Phenobarbital showed similar effects as Fipronil following administration to rats for 14 days, including a statistically significant decrease in the terminal half-life (31%) and increases in the clearance (84%) and volume of distribution (25%). Phenobarbital also appeared to induce some effects upon thyroxine clearance from whole-blood after only 1 day of dosing as indicated by a statistically significant decrease in the terminal half-life (18%) and significant increases in the volume of distribution (23%)	x x
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was administered orally, by gavage, to male Sprague-Dawley CD rats rats in order to assess thyroxine clearance and hence its potential to indirectly affect thyroid function. The rats used were 49 days old upon arrival at the test facility. They were housed in groups of 3 and acclimatised for 4 days before the start of treatment and weighed between 282 and 306 g at the start of dosing. Groups of six male rats were given 1 or 14 consecutive daily oral doses, by gavage, of either 0 or 10 mg/kg bw/d of Fipronil suspended in 0.5% methylcellulose, at a dose volume of 5 ml/kg bw. A similar sized group was given 80 mg/kg bw/d of Phenobarbital, suspended in the same vehicle, at a dose volume of 2 ml/kg as positive control. All dose preparations were prepared freshly each day prior to use and the dose volume was adjusted according to the most recently recorded bodyweight. On Day 1-2 and Day 14-15 of treatment, the dose formulations were analysed to check homogeneity of mixing Fipronil with the vehicle and its stability in this medium after 24 hours of storage at room temperature. Furthermore, dose formulations prepared on Days 1 and 14 were analysed for test material content.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**	
	On Day 1 for those rats given just one daily dose, and on Day 14 for those given 14 daily doses, each rat received two intraperitoneal injections of 1 mg of NaI in 0.9% saline solution (0.5 ml). They were administered about 5 minutes and about 10 hours 5 minutes after the dose of test material. Similarly, on Day 1 or 14, each rat received a dose of [^{125}I]-thyroxine (T_4) in 0.9% saline solution (10 µCi/kg bodyweight) by infusion at a constant dose volume of 2 ml/kg bw 4 hours after test material administration. It was dosed via the tail vein at a rate of 1 ml/minute, the dose being adjusted according to the bodyweight recorded on the previous day. The NaI and [^{125}I)-thyroxine saline solutions were prepared accurately on the day prior to use and stored at 4^{o}C in the dark. All animals were observed twice daily for mortality and at least once daily for clinical signs. Individual was recorded at the start of treatment and prior to termination (for those rats killed after 1 dose) and weekly for those killed after 14 daily doses. Additionally weights were documented on Day -1 and Day 13 in order to calculate the dose volumes for administration of [^{125}I]-thyroxine. Food consumption was measured for each cage group over Days -2 to Day 3 for those rats given a single dose of the test material and from Day -2 to Day 7 and on Day 14 and at termination for those given14 doses. Water intake was monitored by visual appraisal of the water bottles. Blood samples from the tail vein were collected on Day 1 or Day 14 for those rats given 1 or 14 daily doses, respectively. They were taken at 0.5, 1, 2, 4, 8, 12, 20, 24 and 30 hours after administration of [^{125}I]-thyroxine and were used to estimate pharmacokinetic parameters of thyroxine terminal half-life, clearance and volume of distribution. An additional blood sample was taken at termination. They were not examined.	x
5.2 Results and discussion	<u>Analysis of dose preparations</u> Analysis of samples from the nominal concentration of 0.2% w/v of Fipronil dose suspensions prepared for the first and last days of dosing (Day 1 and 14 respectively) showed that they contained 0.17 and 0.14% w/v of test material, respectively. Aliquots from the top, middle and bottom of these same samples retained with stirring at room temperature for 24 hours to assess stability contained 0.23% w/v. Therefore, the achieved concentration of Fipronil and homogeneity of mixing was acceptable and there was no degradation following storage at room temperature at 24 hours. <u>Clinical signs</u> No clinical signs were attributed to treatment with Fipronil. Collapsed posture and lethargy were observed after dosing in all Phenobarbital-treated animals. Shallow breathing was also seen on the first day of dosing. <u>Mortality</u> There were no deaths. <u>Bodyweights, food consumption and efficiency of food utilisation</u> Intergroup differences were considered to be unrelated to treatment with Fipronil or Phenobarbital.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Investigation of thyroid toxicity**	
	Thyroxine (T_4) clearance Thyroxine concentration in whole blood showed a biphasic distribution with time indicative of an absorption phase followed by an excretion/metabolism phase. In the Fipronil-treated groups, no statistically significant effects on thyroxine pharmacokinetic parameters were seen after a single dose. After 14 daily doses, the terminal half-life was significantly decreased (by 48% compared with controls) whilst the clearance and volume of distribution were significantly increased (by 161% and 37%, respectively). A single dose of 80 mg/kg bw/d of Phenobarbital induced a statistically significant decrease in the terminal half-life of Thyroxine (18% below control values), and significant increases in the clearance (32%) and in the volume of distribution (9%). After 14 daily doses, the terminal half-life was decreased (31%) whilst clearance and volume of distribution were increased (84% and 25%, respectively). **Discussion:** Fipronil induced thyroxine clearance from whole blood when given 14 consecutive daily doses of 10 mg/kg bw/d as indicated by increased clearance and volume of distribution compared with controls. These increases were paralleled by a significant decrease in the terminal half-life. Although qualitatively similar, the smaller changes seen after a single dose were not statistically significant. Indeed there was no apparent change in the volume of distribution. In general, the changes observed were similar to those caused by 80 mg/kg bw/d of Phenobarbital. However, there were some differences. For example, a single dose of Phenobarbital induced a significant increase in thyroxine clearance. After 14 daily doses, the magnitude of the changes by Phenobarbital was not as marked as those induced by Fipronil.	
5.3 Conclusion	Fourteen daily oral administration of 10 mg/kg bw/d of Fipronil to male rats stimulated the clearance of Thyroxine (T_4) from whole blood. Fipronil generally acted like Phenobarbital, although appeared to be more potent after 14 consecutive days of administration. No changes in the pharmacokinetic parameters of Thyroxine occurred after a single dose of Fipronil, whereas significant increases in the clearance of Thyroxine were observed only 4 hours after a single dose of 80 mg/kg bw of Phenobarbital.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Figure 6.10.1-1 **Plot of concentration of thyroxine in whole-blood on a logarithmic scale against time of blood sampling following administration of [^{125} I] thyroxine to rats that had been dosed for ~~one day~~ fourteen days (Each point represents the mean value for a group of six animals)**

X

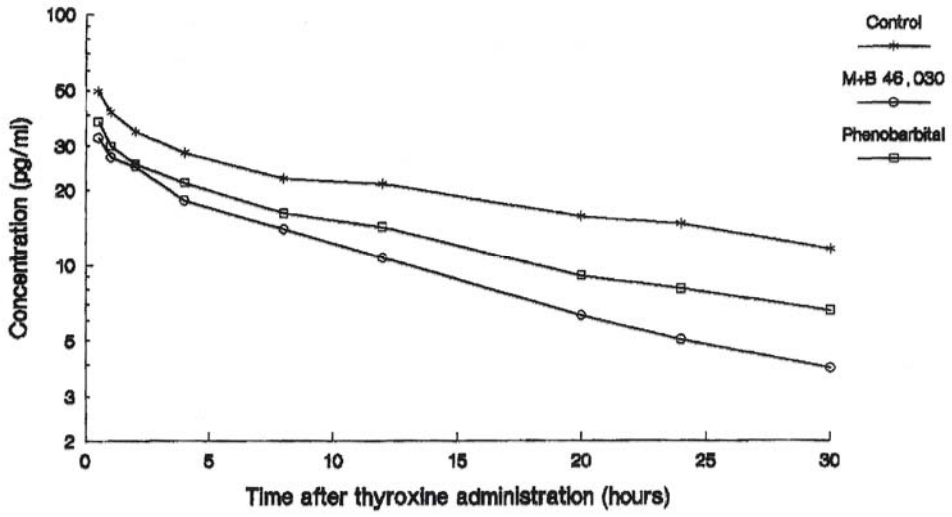

Figure 6.10.1-2 **Plot of concentration of thyroxine in whole-blood on a logarithmic scale against time of blood sampling following administration of [^{125} I] thyroxine to rats that had been dosed for ~~fourteen days~~ one day (each point represents the mean value for a group of six animals)**

X

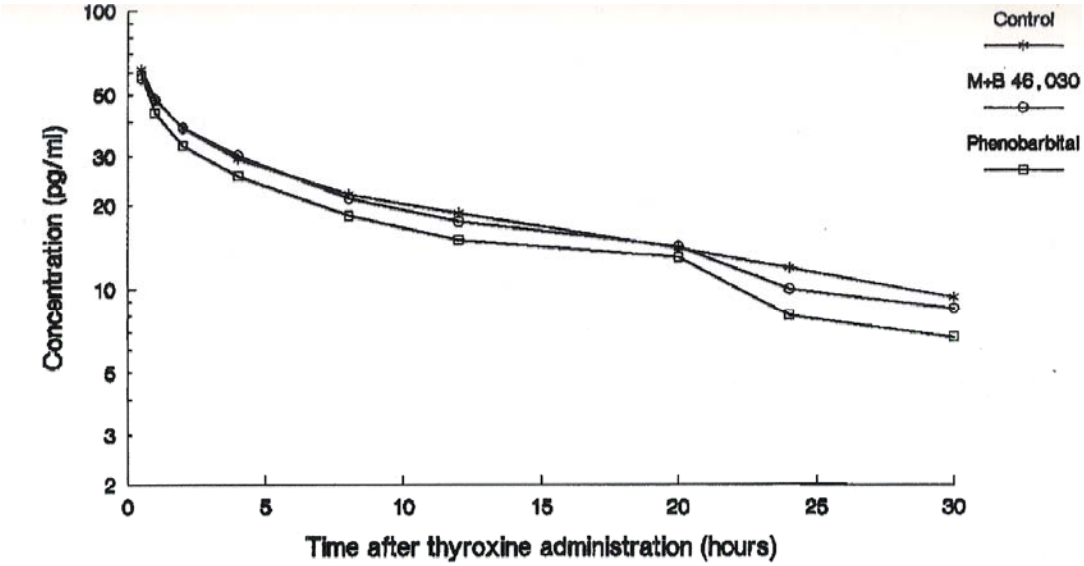

Table A6.10.1-1 Mean concentrations of thyroxine in whole-blood of rats – pg/ml

Test group	Dose (mg/kg bw/d)	Time point of thyroxine measurement (h post [^{125}I] thyroxine application)								
		0.5	1	2	4	8	12	20	24	30
Single treatment										
Control	0	61.49	48.26	38.11	29.45	21.97	18.82	14.11	12.00	9.41
Fiponil	10	57.19	47.98	38.10	30.27	21.33	17.56	14.26	10.05	8.52
Phenobarbital	80	58.59	42.93	32.81	25.50	18.41	15.07	13.04	8.06	6.72
14-day treatment										
Control	0	49.44	40.96	34.25	28.23	22.34	21.28	15.83	14.81	~~11.84~~ 11.74
Fiponil	10	32.35	27.08	24.92	18.26	13.99	10.75	6.31	5.06	3.90
Phenobarbital	80	37.49	30.01	25.54	21.44	16.20	14.33	9.10	8.08	6.62

X

Table A6.10.1-2 Kinetic parameters for thyroxine determined in whole-blood of rats – group mean values

Parameter	1-day treatment			14-day daily treatment		
	Control	Fipronil (10 mg/kg bw)	Phenobarbital (80 mg/kg bw)	Control	Fipronil (10 mg/kg bw/d)	Phenobarbital (80 mg/kg bw/d)
Terminal half life (hours)	17.2	15.6	14.1**	22.5 ± 2.4**	11.8 ± 1.5**	15.5 ± 2.6**
Clearance (ml/min)	0.0548	0.0606	0.0722**	0.0568	0.1484 **	0.1045 **
Volume of distribution (ml)	80.54	80.43	87.83*	110.05 **	150.31 **	137.83 **

Statistical evaluation: * = p<0.05; ** = p<0.01; *** = p<0.001 (Student's t-test). In the case of the control groups, levels of significance are for comparisons of data between Day 14 and Day 1 control group animals.

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.2.4 Sex: *Male, approx.* ~~*49*~~ 53 *days old, weighing between 282–306 g at the start of dosing* 3.4.4 Thyroxine clearance: *Blood was withdrawn 0.5, 1, 2, 4, 8, 12, 20, 24 and* 30 *hours after administration of [^{125}I]thyroxine.*
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.3 Thyroxine Clearance: *These included a highly significant decrease in the terminal half-life* ~~*(45%)*~~ (48%) *and increases in the clearance (161%) and volume of distribution (37%).* *Phenobarbital also appeared to induce some effects upon thyroxine clearance from whole-blood after only 1 day of dosing as indicated by a statistically significant decrease in the terminal half-life (18%) and significant increases in the volume of distribution* ~~*(23%)*~~ (9%) and in the clearance (32%).
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Individual* bodyweight *was recorded at the start of treatment and prior to termination (for those rats killed after 1 dose) and weekly for those killed after 14 daily doses.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**

		1. REFERENCE	**Official use only**
1.1	**Reference**	**A6.10/02** XXXX M&B 46,030: An investigation into the potential effects on thyroid function in male rats using the "Perchlorate Discharge Test". XXXX (including 2-page amendment from 1993). (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No No guideline exists for this mechanistic investigation	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	**X**
3.1.1	Lot/Batch number	PGS 963	
3.1.2	Specification	As given in Section 2	**X**
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	95.4 %	
3.1.2.3	Stability	Stable	**X**
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley (Crl: CD(SD))	
3.2.3	Source	XXXX	
3.2.4	Sex	Male, approximately 42 days old, weighing between 260 -306	**X**
3.2.5	Number of animals per group	27	
3.2.6	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	14 days	
3.3.2	Frequency of exposure	Once daily	
3.3.3	Postexposure period	1 day	
3.3.4	**Oral**		
3.3.4.1	Type	Gavage	
3.3.4.2	Dose	10 mg/kg bw/d	
3.3.4.3	Vehicle	0.5% aqueous methylcellulose	
3.3.4.4	Concentration in vehicle	2 mg/ml	
3.3.4.5	Total volume applied	5 ml/kg bw/d	
3.3.4.6	Controls	Vehicle control: 0.5% aqueous methylcellulose Positive control: Propylthiouracil, 200 mg/kg bw/d (oral gavage, 5 ml/kg bw, in 0.5% aqueous methylcellulose)	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
3.3.4.7 Experimental design	Positive control: Noxyflex, 50 mg/kg bw/d (intraperitoneal, 1 ml/kg bw/d in 0.9% aqueous saline solution) Groups of 27 rats were administered either the vehicle (0.5% methylcellulose), 10 mg/kg bw/d Fipronil or as positive control 200 mg/kg bw/d Propylthiouracil (PTU) daily for 14 days by oral gavage. A second positive control group of 27 rats received daily i.p. injections of 50 mg/kg bw/d Noxyflex for 14 days. On Day 15, approx. 24 hours after the last treatment, each animal received 1 µCi Na[^{125}]I in 0.5 ml 0.9% saline by i.p. injection. Six hours later, subgroups of 9 males from each dose group received 10 mg/kg bw or 25 mg/kg bw potassium perchlorate or the vehicle (0.9% aqueous saline) by i.p. injection. Immediately after i.p. administration of potassium perchlorate or saline solution, each surviving rat was anaesthetized, and approx. 2 ml blood was obtained by cardiac puncture for radioactivity analysis. Rats were subsequently killed by CO_2 asphyxiation, the thyroid gland was removed, weighed and processed for radioactivity analysis.	
3.4 Examinations		
3.4.1 Clinical signs and mortality	Check for mortality and moribund animals: twice daily, 7 days/week Check for any signs of behavioural changes, reaction to treatment or ill health: at least once daily except on Days 14 and 15 of treatment	
3.4.2 Bodyweight	Determined upon group allocation, on treatment day 1 and weekly thereafter.	
3.4.3 Food consumption	Determined weekly (g/rat/week, cage-wise calculation, 3 rats/cage) Food conversion ratios were calculated from bodyweight and food consumption data as weight of food consumed per unit gain in body weight	
3.4.4 Formulation sampling	Stability, homogeneity and concentration control determinations of fipronil preparations were conducted on samples obtained on Day 1 and Day 14 of the study.	
3.4.5 Terminal examinations	<u>Blood sampling:</u> On day 15, 2 ml blood was obtained from each rat by cardiac puncture and processed for radioactivity analysis. <u>Necroscopy:</u> The thyroid gland was removed, rinsed with saline, blotted dry, weighed and subjected to radioactivity analysis. All animals were discarded without further examination. <u>Radioactivity determinations in whole blood and thyroid:</u> Levels of radioactivity in pairs of thyroid glands and duplicate 0.5 g aliquots of whole blood were estimated by gamma counting. The percentage efficiency of counting of samples on the gamma counter was estimated by the counting of a gamma source (^{129}I) of known activity (182,040 dpm).	
	4. RESULTS AND DISCUSSION	
4.1 Mortality	One rat treated with 50 mg/kg bw/d Noxyflex was found dead on Day 2 of the study. No previous signs of ill health, behavioural change or reaction to treatment were noted.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
4.2 Clinical Signs	Fipronil or Noxyflex: No clinical findings indicative of a reaction to treatment with Fipronil or Noxyflex were noted. Propylthiouracil (PTU): Salivation was noted in all rats immediately after administration of PTU, which was mainly first noted during the first treatment week. Salivation occasionally persisted up to approx. 1 hour after dosing. In addition, a low incidence of brown peri-oral staining immediately after dosing and matted fur up to approx. 30 min after dosing. 2 rats were found with wet urogenital regions.	
4.3 Bodyweight	Fipronil, Noxyflex, PTU: Statistically significant reduction in body weight gain within 14 days for Fipronil (-13%***), PTU (-62%***), and Noxyflex (-23%***) (See Table A6.10.2-1)	**x**
4.3 Food consumption	Fipronil, Noxyflex, PTU (see Table A6.10.2-2) Statistically significant reduction in food intake during the second treatment week for Fipronil (-8%**), PTU (-27%***), and Noxyflex (-14%***). The efficiency of food utilisation by rats administered Fipronil or Noxyflex was essentially similar to control levels. PTU treated rats showed a reduced food utilisation effeciency especially during the second treatment week (due to marked reduction in body weight gain but less marked reduction of food intake) No overt treatment group differences of water consumption were noted.	
.4 Levels of radioactivity in whole blood	(See Table A6.10.2-3) Increased radioactivity levels [^{125}]I in whole blood were found after 14-day treatment of rats with PTU (+43%) or with Noxyflex (+13%) and without subsequent potassium perchlorate administration. No notable change in radioactivity levels were found after Fipronil treatment. Potassium perchlorate treatment had no statistically significant effect on blood levels. There was a small increase in PTU-treated rats and a small decrease in Noxyflex-treated rats upon treatment with 10 mg/kg bw/d potassium perchlorate. Effects seen after administration of 25 mg/kg bw/d potassium perchlorate were smaller than after administration of 10 mg/kg bw/d potassium perchlorate.	**x**
4.5 Level of radioactivity in thyroids	(See Table A6.10.2-4) Statistically significant increases in thyroid radioactivity levels (^{125}I) were found after 14-day treatment of rats with Fipronil or with Noxyflex. A statistically significant reduction in radioactivity levels in thyroids were found after PTU treatment. In PTU groups subjected to 10 or 25 mg/kg bw potassium perchlorate treatment, a pronounced further decrease in thyroid radioactivity levels was seen. In groups administered Fipronil or Noxyflex, potassium perchlorate challenge did not lead to a decrease of radioactivity in the thyroid.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
4.6 Thyroid weights	(See Table A6.10.2-5) In groups treated with Fipronil, PTU or Noxyflex a statistically significant increase in thyroid weights was observed. PTU caused the most marked effect, compared with Fipronil or Noxyflex. Treatment with potassium perchlorate had no impact on thyroid weight.	
4.7 Ratio of blood radioactivity : thyroid weight	PTU treatment resulted in a decrease of the blood radioactivity level relative to thyroid weight. This decrease was caused by the increase in thyroid weight of PTU-treated rats, which was more pronounced than the increase of whole blood radioactivity levels. Since Fipronil treatment had no effect on whole blood radioactivity levels but caused a significant thyroid weight increase, the composite blood radioactivity relative to thyroid weight was decreased, (incidentally) reaching statistical significance in the Fipronil subset group administered 25 mg/kg bw potassium perchlorate. A similar finding was observed for Noxyflex-treatment group rats subjected to 25 mg/kg bw potassium perchlorate.	
4.8 Ratio of blood radioactivity : thyroid radioactivity	(See Table A6.10.2-6) Fipronil or Noxyflex treatment resulted in an <u>increased</u> ^{125}I-concentration thyroid/whole blood ratio (by 59 and 58%, respectively), compared to the saline control. By contrast, Propylthiouracil treatment led to a <u>reduction</u> in the ^{125}I-concentration thyroid/whole blood ratio (in agreement with an ^{125}I increase in whole blood and a ^{125}I decrease in the thyroid, as reported). Treatment with potassium perchlorate enhanced this reduction in the Propylthiouracil-treated group (up to 99%), which reflected efflux of free ^{125}I from the thyroid. By contrast, in groups administered Fipronil or Noxyflex, potassium perchlorate treatment did not lead to a decreased thyroid/whole blood radiolabel ratio.	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** This mechanistic study was conducted to investigate whether Fipronil exerts a direct toxic effect on the thyroid by inhibiting the synthesis of thyroid hormones T_3 and T_4. In the "Perchlorate Discharge Test", radiolabelled iodide is offered as substrate to rats previously exposed to the test or control substance. Test compounds may inhibit the thyroid peroxidase, which catalyses the stepwise binding of iodide to the tyrosyl residues of thyroglobulin and thus results in less thyroid-bound iodide. Bound and free radioactive iodide present in the thyroid can be distinguished by a challenge treatment with potassium perchlorate, which competes with free iodide for active transport into the thyroid follicular lumen, and causes the liberation of free iodide from the thyroid into the blood. In animals offered radiolabelled iodide after treatment with test compounds, which do not inhibit organification, there is no depletion of radioactivity from the thyroid following perchlorate treatment. Fipronil was administered by gavage to male Sprague-Dawley CD rats. The rats were approximately 42 days old at arrival and weighed between 260 and 306 g at the start of dosing. They were housed in groups of 3 and were acclimatised for 5 days before treatment began.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
	Four groups of 27 male Sprague-Dawley rats were used. Two groups were given 14 consecutive daily oral doses, by gavage, of either 0 or 10 mg/kg bw/d of Fipronil suspended in 0.5% Methylcellulose and administered at a dose volume of 5 ml/kg. A third similar-sized group received fourteen daily gavage doses of 200 mg/kg bw/d of Propylthiouracil (PTU), an inhibitor of thyroid iodide organification. It was suspended in the same vehicle and given at the same dose volume. The fourth group was given 14 daily intraperitoneal injections of 50 mg/kg bw/d of Noxyflex, a putative inhibitor of thyroid iodide organification. This was dissolved in 0.9% saline and administered at a dose volume of 1 ml/kg. All dose preparations were prepared freshly each day prior to use. Dose volumes were adjusted according to the most recently recorded bodyweight. Dose preparations of Fipronil prepared for Day 1 and Day 14 were analysed for achieved concentration of the test material, its homogeneity of mixing with the vehicle and stability in this medium. On Day 15 of the study and 24 hours after the last dose, each rat received a single intraperitoneal injection of radiolabelled $Na^{125}I$ (1 µCi/kg bodyweight) in 0.5 ml 0.9% w/v saline solution. The dose was prepared the day prior to use. Six hours later, 9 males from each group received an intraperitoneal injection of 25 mg/kg of potassium perchlorate in 0.9% saline. All rats were observed twice daily for mortality and once daily for clinical signs. Individual bodyweights were recorded at the start of treatment and weekly thereafter. Food consumed by each cage group was measured at weekly intervals during the treatment period whilst water intake was monitored by visual inspection of the water bottles. At termination on Day 15, immediately after the intraperitoneal injection of potassium perchlorate, each surviving rat received an intramuscular injection of a neuroleptanalgesic. Two and a half minutes later about 2 ml was withdrawn by cardiac puncture. The rats were then killed immediately and the thyroid dissected and weighed. Radioactivity levels in the thyroid and whole blood were subsequently quantified.	**X**
5.2 Results and discussion	<u>Analysis of dose preparations</u> Analysis of samples from the nominal concentration of 0.2% w/v of Fipronil dose suspensions prepared for the first and last days of dosing (Day 1 and 14 respectively) showed that they contained 0.19 and 0.16% w/v of test material, respectively. Aliquots from the top, middle and bottom of these same samples retained with stirring at room temperature for 24 hours to assess stability contained 0.22% w/v. Therefore, the achieved concentration of Fipronil and homogeneity of mixing was acceptable and there was no degradation following storage.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
	Mortality There were no deaths in the groups treated with Fipronil or Propylthiouracil. One rat given 50 mg/kg bw/d of Noxyflex was found dead on Day 2. No clinical signs were observed prior to death. Clinical signs No clinical signs were attributed to treatment with Fipronil or Noxyflex. In the Propylthiouracil-treated rats, salivation was seen in all animals immediately after dosing and occasionally persisted for up to 1 hour post-dosing, mainly in Week 1. Lower incidences of brown peri-oral staining immediately post-dosing and of matted fur up to about 0.5 hours after dosing were also noted. Two rats exhibited a wet urogenital region during Week 2. Bodyweight (see Table A6.10.2-1) Bodyweight gain was reduced in all groups of treated rats and was marked in animals given propylthiouracil, particularly during the second week of treatment. Food consumption (see Table A6.10.2-2) Food consumption was reduced in all treated groups compared with controls. It was marked in animals dosed with propylthiouracil where it was mainly due to inappetance during the second week of treatment. Food conversion efficiency Overall food efficiency was only reduced in rats treated with Propylthiouracil, particularly during the second week of treatment. Food conversion efficiency was unaffected in rats given Fipronil or Noxyflex, reflecting similar reductions in bodyweight gain and food intake. Water consumption There was no overt treatment-related effect. Radioactivity levels in whole blood (see Table A6.10.2-3) Treatment with Fipronil or Noxyflex had no significant effect. In the Propylthiouracil-treated group, however, increased concentrations (+43%) of ^{125}I in whole blood were found compared with the saline controls; administration of 10 mg/kg of potassium perchlorate resulted in a further increase in whole blood radioactivity, which was considered to be due to an efflux of free ^{125}I from the thyroid. Radioactivity levels in thyroid (see Table A6.10.2-4) Increased ^{125}I accumulation in the thyroid was observed in the Fipronil- and Noxyflex-treated groups (+80% and +106%, respectively). In contrast, ^{125}I accumulation was reduced by 55% in the Propylthiouracil-treated group. Administration of 10 mg/kg of Potassium perchlorate resulted in an efflux of ^{125}I from the thyroid in this Propylthiouracil-treated group. Thyroid weights (see Table A6.10.2-5) Group mean thyroid weight was markedly increased in the propylthiouracil-treated group (+174% of controls). Marginal increases were also found in rats given Fipronil or Noxyflex (+8% and +19%, respectively).	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Perchlorate discharge test**	
	Thyroid radioactivity : whole blood radioactivity ratio (see Table A6.10.2-6) Fipronil or Noxyflex treatment resulted in an increased ^{125}I-concentration thyroid/whole blood ratio (by 59 and 58%, respectively), compared to the saline control. By contrast, Propylthiouracil treatment led to a reduction in the ^{125}I-concentration thyroid/whole blood ratio (in agreement with an ^{125}I increase in whole blood and a ^{125}I decrease in the thyroid, as reported). Treatment with potassium perchlorate enhanced this reduction in the Propylthiouracil-treated group (up to 99%), which reflected efflux of free ^{125}I from the thyroid. By contrast, in groups administered Fipronil or Noxyflex, potassium perchlorate treatment did not lead to a decreased thyroid/whole blood radiolabel ratio. The increased ratio seen in Fipronil and Noxyflex-treated animals can be explained by an increased thyroid hormone production in the thyroid (reflecting increased Iodine demand and therefore increased ^{125}I-levels in the thyroid) to compensate decreased whole blood levels of thyroid hormones. ^{125}I present in the thyroid in Fipronil-treated animals is not displaced following treatment with perchlorate, indicating that organification of iodine is not impacted by Fipronil treatment. **Discussion:** This study investigated the potential of Fipronil on the thyroid function of male rats. Its activity was compared with that of propylthiouracil, a known inhibitor of thyroid organification in many species. It was also compared with that of Noxyflex (Noxythiolin), another thiourea, which has been shown to lower serum thyroxine levels in rats and to reduce iodide organification in cultured porcine thyrocytes in vitro following 14 days of treatment. Fourteen consecutive daily oral doses of 10 mg/kg bw/d of Fipronil, stimulated follicular activity in the thyroid as evidenced by increased accumulation of ^{125}I in this gland and by increases in the ratio of radioactivity between the thyroid and blood. There was also a small increase in thyroid weight in these rats. Similar responses were induced by 14 daily intraperitoneal injections of 50 mg/kg bw/d of Noxyflex.	

Section A6.10	**Mechanistic studies**
Annex Point IIIA, VI.7	**- Perchlorate discharge test**

	Daily oral administration of 200 mg/kg bw/d of propylthiouracil (PTU) for 14 consecutive days resulted in a marked statistically significant decrease in the amount of ^{125}I incorporated into the thyroid and in the:thyroid/blood ratio. These responses were reflected by increased levels of ^{125}I in whole blood. Thyroid weights were increased by more than 2.5-fold compared with controls, otherwise ^{125}I-levels would have been even higher after PTU treatment. This was demonstrated by expressing blood concentrations as a function of thyroid weight, which resulted in a reduction of "corrected" ^{125}I concentration in whole blood of about 50% for PTU compared to control levels (data given in the study report). Propylthiouracil is believed to act by inhibiting thyroid peroxidase activity, the enzyme responsible for incorporating iodide into thyroglobulin. Therefore, whilst uptake of iodide is not impaired, its retention is reduced and organification is blocked. Concomitantly, pituitary TSH release is activated by feedback which, on sustained inhibition of thyroxine synthesis, stimulates the thyroid resulting in its functional hyperactivity and growth. This lattermost activity can be further demonstrated in the perchlorate discharge study in which any free iodide is released on administration of potassium perchlorate. Two concentrations of potassium perchlorate were given to subgroups of the Fipronil-, Noxyflex- and propylthiouracil-treated groups. Additional large reductions in the ^{125}I content of the thyroid and in the thyroid:blood ^{125}I radioactivity ratios were found in the propylthiouracil-treated rats given perchlorate. The marked efflux of ^{125}I from the thyroid after treatment with potassium perchlorate is consistent with Propylthiouracil's ability to inhibit iodide organification. No evidence of an inhibition of iodide organification was induced by either Fipronil or Noxyflex at the dose levels used. Both of these compounds enhanced the accumulation of radioiodide by the thyroid, obviously triggered by stimulation of its functional activity through TSH stimulation. Therefore this activity must be triggered by a mechanism other than inhibition of iodide organification. It is unclear whether higher Noxyflex dose level would have confirmed the inhibition of organification that had been shown under in-vitro cell culture conditions.	
5.3 Conclusion	Fourteen consecutive daily doses of 10 mg/kg bw/d of Fipronil to male rats stimulated thyroid follicular activity as evidenced by slightly increased thyroid weight and increased thyroid ^{125}I accumulation. However, there was no evidence of an inhibition of the organification of iodide since no efflux of thyroidal ^{125}I was seen after potassium perchlorate administration.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

Table A6.10.2-1 Group mean bodyweight (g) and bodyweight gain

Week	Dose Level (mg/kg/day)			
	Fipronil		PTU	Noxyflex
	0	10	200	50
Body weight				
0	287	287	288	287
1	343	328	324	331
2	389	373	326	363
Body weight gain				
0-2 (% of control)	102 (100)	87*** (85)	38*** (37)	77*** (75)

PTU: Propylthiouracil; Statistical evaluation: *** p<0.001

Table A6.10.2-2 Group mean food consumption (g/rat)

Week	Dose level (mg/kg bw/d)			
	Fipronil		PTU	Noxyflex
	0	10	200	50
1	241	215	197	209
2	233	221	149	201
Total 1-2	474	436**	347***	410***
(% of control)	(100)	(92)	(73)	(86)

PTU: Propylthiouracil; Statistical evaluation: ** p<0.01; *** p<0.001

Table A6.10.2-3 Concentration of I^{152} in whole blood (% of total dose)

Group	Dose level (mg/kg bw/d)			
	Fipronil		PTU	Noxyflex
	0	10	200	50
Saline (% of control)	0.142 (100%)	0.147 (104%)	0.203 (143%)	0.160 (113%)
10 mg/kg bw Perchlorate (% of control)	0.131 (100%)	0.143 (109%)	0.229 (175%)	0.142 (108%)
25 mg/kg bw Perchlorate (% of control)	0.145 (100%)	0.142 (98%)	0.214 (148%)	0.155 (107%)
Overall group means (% of control)	0.139 (100%)	0.144 (104%)	0.215** (155%)	0.152 (109%)

PTU: Propylthiouracil; statistical evaluation: ~~** = p < 0.05~~ ** = p < 0.01

X

Table A6.10.2-4 Radioactivity in thyroids (% of total dose)

Group	Dose level (mg/kg bw/d)			
	Fipronil		PTU	Noxyflex
	0	10	200	50
Saline (% of control)	3.078 (100)	5.541** (180)	1.388** (45)	6.355** (206)
10 mg/kg bw Perchlorate (% of control)	3.191 (100)	5.245** (164)	0.316** (10)	7.267** (228)
25 mg/kg bw Perchlorate (% of control)	2.795 (100)	7.086** (254)	0.237** (8)	7.777** (278)

PTU: Propylthiouracil; Statistical evaluation: ** p<0.01

Table A6.10.2-5 Thyroid weight (g)

Group	Dose level (mg/kg bw/d)			
	Fipronil		PTU	Noxyflex
	0	10	200	50
Saline	0.0189	0.0205	0.0518	0.0224
(% of control)	(100)	(108)	(274)	(119)
10 mg/kg bw Perchlorate	0.0178	0.0175	0.0520	0.0235
(% of control)	(100)	(98)	(292)	(132)
25 mg/kg bw Perchlorate	0.0164	0.0248	0.0594	0.0238
(% of control)	(100)	(151)	(362)	(145)
Overall group mean	**0.017** **(100)**	**0.021**** **(124)**	**0.054**** **(318)**	**0.023**** **(135)**

PTU: Propylthiouracil; statistical evaluation: ** p<0.01

Table A6.10.2-6 Ratio of ^{125}I concentration in thyroid to that in whole blood

Group	Dose level (mg/kg bw/d)			
	Saline	Fipronil	PTU	Noxyflex
	0	10	200	50
Saline (% of control)	1150.7 (100)	1833.9** (159)	133.8* (12)	1821.3** (158)
10 mg/kg bw Perchlorate (% of control)	1423.1 (100)	2140.7** (150)	26.5** (2)	2217.8** (156)
25 mg/kg bw Perchlorate (% of control)	1307.4 (100)	2053.3** (157)	19.2** (1)	2164.5** (166)

PTU: Propylthiouracil; Statistical evaluation: * p<0.05; ** p<0.01

X

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.2.4 Sex: *Male, approximately* ~~*42*~~ 47 *days old, weighing between 260 –306* at the start of dosing
Results and discussion	Agree with applicant's version. **Revisions/amendments:** 4.3 Bodyweight: *Statistically significant reduction in body weight gain within 14 days for Fipronil* ~~*(13%*~~ -15%***), *PTU* ~~*(62%*~~ -63%***), *and Noxyflex* ~~*(23%*~~ -25***) ~~*.4 Levels of radioactivity in whole blood*~~ 4.4 Levels of radioactivity in whole blood
Conclusion	Agree with applicant's version. **Revisions/amendments:** 5.1 Materials and methods: *Two and a half minutes later about 2 ml* of blood *was withdrawn by cardiac puncture.*
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.10	**Mechanistic studies**
Annex Point IIIA, VI.7	**- Rat biliary excretion of radiolabelled thyroxine**

			Official use only
		1. REFERENCE	
1.1	**Reference**	**A6.10/03** 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 XXXX; (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 to Annex 1	
		2. GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No No guideline exists for this type of mechanistic study	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3. MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2	X
3.1.1	Lot/Batch number	78/GC/90	
3.1.2	Specification	As given in Section 2	X
3.1.2.1	Description	White powder	X
3.1.2.2	Purity	96.7%	X
3.1.2.3	Stability	Stable	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley [Crl: CD(SD)]	X
3.2.3	Source	XXXX	
3.2.4	Sex	Male, approximately 6 -8 weeks old, weighing about 200 g at the start of the study	
3.2.5	Number of animals per group	3	
3.2.6	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	a) 1 day b) 14 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Postexposure period	Approx. 9 hours (biliary excretion examined for 5 hours, starting 4 hours after last application of the test compound)	
3.3.4	**Oral**		
3.3.4.1	Type	Oral gavage	
3.3.4.2	Dose	1 and 10 mg/kg bw/d Fipronil	
3.3.4.3	Vehicle	0.5 % aqueous methylcellulose	
3.3.4.4	Concentration in vehicle	0.2 and 2 mg/ml Fipronil	
3.3.4.5	Total volume applied	5 ml/kg bw	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Rat biliary excretion of radiolabelled thyroxine**	
3.3.4.6 Controls	Negative control: Vehicle (0.5 % aqueous methylcellulose, oral gavage) Positive control: Phenobarbital (i.p. dose: 80 mg/kg bw/d, 32 mg/ml, aqueous dilution)	
3.3.4.7 Experimental design	Groups of 3 rats were administered 1 or 10 mg/kg bw/d Fipronil in 0.5% aqueous methylcellulose by oral gavage for 1 or 14 days. A negative control group received the vehicle (0.5 % aqueous methylcellulose). A positive control group received 80 mg/kg bw/d Phenobarbital dissolved in distilled water by intraperitoneal injection either for 1 or daily for 14 days. Immediately after the last (or single) administration of test substance, the bile duct of the animals was cannulated under halothane anaesthesia. As a measure to prevent uptake of ^{125}I by the thyroid, 1 mg sodium iodide was subsequently injected directly into the stomach. 4 hours after the last administration of test or control substance, approx. 10 µCi ^{125}I-thyroxine was given by intravenous injection to each animal. Bile was collected prior to dosing and sampled at 15 min intervals during 0–5 hours following administration of ^{125}I-T_4. 10 µl blood samples were taken from the tail vein at 30-minute intervals during 0–5 hours following administration of ^{125}I-T_4. At 5 hours rats were killed and the livers removed.	
3.4 Examinations		
3.4.1 Necroscopy examinations	Terminal body weights and liver weights were determined for each animal.	
3.4.2 Analysis bile, whole blood and liver samples	All bile, whole blood and liver samples were analysed for total radioactivity. Selected bile samples were analysed by HPLC before and after treatment with ß-glucuronidase.	
	4. RESULTS AND DISCUSSION	
4.1 Liver weight and bile weight	(See Tables A6.10.3-1 and -2) After a single dose of either Fipronil at 1 or 10 mg/kg bw or Phenobarbital at 80 mg/kg bw, there were no significant differences from control in liver weights adjusted for bodyweight, or in weight of bile excreted during 0 – 5 hours. After 14 days of dosing, the Phenobarbital group ($p < 0.001$) and high dose Fipronil group ($p < 0.01$) excreted significantiy higher weights of bile during 5 hours than the control group. For liver weight adjusted for bodyweight, only the high-dose Fipronil group showed significantly higher values than control ($P < 0.05$).	
4.2 Results of radioactivity analysis in bile, whole blood and liver	<u>Single-dose treatment</u> (see Table A6.10.3-3) Following administration of single doses of 1 or 10 mg/kg bw Fipronil, 80 mg/kg bw Phenobarbital and 0.5% methylcellulose (Control), cumulative mean totals of 7.55%, 8.61%, 9.34% and 6.48%, respectively, of the intravenous dose of ^{125}I-thyroxine (total radioactivity) were excreted in bile during 0 – 5 hours. Excretion of radioactivity in bile was significantly higher ($P<0.05$) in the Phenobarbital group only, although there were slight increases which were not significantly different for both Fipronil dosing groups.	

Section A6.10	**Mechanistic studies**
Annex Point IIIA, VI.7	**- Rat biliary excretion of radiolabelled thyroxine**

		At *post mortem* only a small proportion of the dose (~10%) was found in the livers (all treatments), with respectively, mean totals of 9.33%, 9.38%, 8.07% and 8.42%. There were no statistically significant differences between any treatment group and control. In whole-blood at 5 hours the mean totals were respectively, 19.28%, 17.67%, 12.95% and 17.45%. Only the Phenobarbital group was significantly lower than control ($p < 0.01$). Thus, after single-dose treatment the mean total percentages of dose in bile, liver and whole-blood were 36.14% (1 mg/kg bw Fipronil), 35.65% (10 mg/kg bw Fipronil), 30.37% (80 mg/kg bw Phenobarbital) and 32.35%. (0.5% methylcellulose)	
4.2	**Results of radioactivity analysis in bile, whole blood and liver** (cont'd)	14-day treatment (see Tables A6.10.3-3 and -4) Following 14 repeated daily doses of each of the above treatments, the cumulative mean totals of 10.98%, 13.58%, 14.88 % and 3.78% respectively of the intravenous dose of ^{125}I-thyroxine (total radioactivity) were excreted in bile during 0–5 hours. All treatment group totals were significantly higher than control ($p<0.001$). Data for the biliary excretion of radioactivity, expressed as ng equivalents ^{125}I-T_4, show that repeated dosing for 14 days with either Fipronil or Phenobarbital increased ca. 3-fold the amount of radioactivity excreted in bile compared with control animals. In whole-blood the mean total proportions were respectively, 12.14%, 8.40%, 12.47% and 8.48%. Comparative analyses of kinetics in whole blood and bile imply that biliary excretion of radioactivity was constant during the 5-hour collection period and was independent of the blood radioactive concentration. Less than 10% of the dose was found in livers taken from all animals at *post mortem* except for those administered Phenobarbital for 14 days; the mean totals were respectively 8.23%, 7.50%, 12.75% and 4.58% for animals treated with 1 mg/kg bw/d Fipronil, 10 mg/kg bw/d Fipronil, 80 mg/kg bw/d Phenobarbital and 0.5 % Methylcellulose (Control). The total percentage of the administered dose in bile, liver and whole-blood was respectively 31.34%, 29.48%, 40.10% and 16.84%.	
4.3	**Biliary clearance**	Single-dose treatment (see Table A6.10.3-5) The mean biliary clearance of radioactivity after single doses of 1 and 10 mg/kg bw Fipronil (1.0 ml/h) was similar to that of control animals (0.8 ml/h). For the Phenobarbital group, the biliary clearance tended to be higher (1.6 ml/h), but this increase was not statistically significant. 14-day treatment (see Table A6.10.3-5) Following 14 repeated single daily doses of Fipronil (1 mg/kg bw/d or 10 mg/kg bw/d) and Phenobarbital (80 mg/kg bw/d) administered to rats, the biliary clearance of radioactivity was ca. 1.5-fold, 4-fold and 2-fold higher, respectively, than that of control animals. This increase was significant for the high dose Fipronil group ($P < 0.001$). Evaluation of blood radioactivity concentration-time profiles on Days 1 and 14, and biliary excretion rates revealed that the decline in blood radioactivity concentrations and biliary excretion with time tended to be parallel with respect to the various treatments.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Rat biliary excretion of radiolabelled thyroxine**	
4.4 Characterisation of radioactivity in bile samples	Analysis of untreated, pooled bile samples by HPLC showed there was very little or no unconjugated [^{125}I]-Thyroxine (T_4) in the samples obtained after either single or repeated doses of any of the treatments. Most of the radioactivity in pooled bile samples (>80%) did not correspond to either free [^{125}I]-iodide or [^{125}I]-T_4. This is in general agreement with the literature (Korhle et al. 1987; Capen, 1989; Cavalieri and Pitt-Rivers, 1981; McClain, 1989). Treatment of pooled bile samples with ß-glucuronidase (also containing sulphatase activity) increased the amount of free ^{125}-T4 and proportionately decreased the amounts of radioactive material, which corresponded neither to free ^{125}I-iodide nor to ^{125}-T_4. The proportions of free ^{125}I-T_4 in pooled bile samples for each treatment group following incubation with ß-glucuronidase indicate that in most instances ca. 48 % or more of the radioactivity in bile corresponded to conjugated ^{125}I-T_4. Single-dose treatment (see Table A6.10.3-6) In the single dose study the biliary excretion of conjugated ^{125}I-T_4 during 0 – 5 hours was about 48 % higher than control (20.2 ng T_4) following treatment with the lower dose (1 mg/kg bw) of Fipronil (29.8 ng T_4), 56% greater than control with treatment at the higher dose (10 mg/kg bw) of Fipronil (31.5 ng T_4) and 74% greater than control after Phenobarbital treatment (35.0 ng T_4). 14-day treatment (see Table A6.10.3-6) In the multiple dose study the excretion of conjugated ^{125}I-T_4 during 0–5 hours was increased ca. 5-fold after Phenobarbital treatment, ca. 3-fold at the lower dose of Fipronil (1 mg/kg bw/d) and ca. 4-fold at the higher dose of Fipronil (10 mg/kg bw/d). These increases over the control levels after multiple doses were greater than that of the corresponding increases after the single doses. Although it was not possible to calculate blood clearance, the pattern of enhanced biliary clearance of ^{125}I-T_4 after treatment with Fipronil and Phenobarbital, appear to correlate with enhanced blood thyroxine clearance observed in a previous study that was conducted with intact, non-bile duct cannulated rats (see Peters et al. 1991, summarised in chapter A6.10.1).	
5.1 Materials and methods	**5. APPLICANT'S SUMMARY AND CONCLUSION** Fipronil was administered to rats in order to assess biliary excretion of intravenously administered ^{125}I-thyroxine after bile duct cannulation. The rats were about 6-8 weeks old and weighed about 200 g at the start of the study. They were housed in groups of three and acclimatised for about 3-4 days before treatment. Groups of three male Sprague-Dawley CD rats were given either 1 or 14 consecutive daily oral doses, by gavage, of 0, 1 or 10 mg/kg bw/d of Fipronil in 0.5% w/v Methylcellulose at a dose volume 5 ml/kg bw. A similar-sized group was given 1 or 14 daily intraperitoneal injections of 80 mg/kg bw/dof Phenobarbital, dissolved in distilled water, as the positive control.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Rat biliary excretion of radiolabelled thyroxine**	
	After the last (or only) dose, the rats were anaesthetised and a cannula surgically implanted in the upper part of the common bile duct for bile collection. A single dose of 1 mg of Sodium iodide, dissolved in water (4 mg/ml), was then administered directly into the stomach. Four hours later, each animal was given an intravenous injection of 10 µCi of ^{125}I-Thyroxine (^{125}I-T_4) in isotonic saline. Samples of bile were collected prior to dosing and at 15-minute intervals during 0-5 hour period after ^{125}I -T_4 administration, weighed and analysed for radioactivity. They were pooled (into groups representing outputs during 0-1, 1-2, 2-3, 3-4 and 4-5 hours post ^{125}I-T_4 administration) and incubated with β-glucuronidase in a water bath for 4 hours at 37°C. The sample was then analysed by HPLC. Blood samples were collected at 30-minute intervals between 0 to 5 hours post-dosing with ^{125}I-T_4. Five 5 hours after ^{125}I-T_4 administration, the rats were killed. Their livers were excised, weighed, homogenised with water and the radioactivity quantified using a gamma scintillation counter.	
5.2 Results and discussion	<u>Radioactivity in whole blood</u> (see Table A.6.10.3-3) Following a single dose of Fipronil or Phenobarbital, only radioactivity in the whole blood samples of the positive controls was lower than in controls. After 14 consecutive daily doses, radioactivity in blood from the 10 mg/kg bw/d Fipronil group rats was comparable to the control values, while the radioactivity in whole blood from 1 mg/kg bw/d Fipronil-treated rats and from the Phenobarbital group was significantly higher than in controls. <u>Radioactivity in liver</u> (see Table A.6.10.3-3) In the liver, only a small proportion (≤10% of the dose administered) was found in all groups, including the control, after a single dose. After 14 consecutive daily doses, a similar proportion of radioactivity was detected in the treated groups while less than 5% was found in controls. (*See Table A.6.10.3-3*) <u>Radioactivity in bile</u> (see Table A.6.10.3-3) After a single dose, a significantly higher amount of radioactivity was observed in bile of the Phenobarbital-treated rats. Although values for the Fipronil-treated groups were slightly higher than controls, statistical significance was not achieved. Following 14 consecutive daily doses, radioactivity levels in bile of all treatment groups were significantly increased over controls: 1 mg/kg bw/d Fipronil (approx. 3-fold), 10 mg/kg bw/d Fipronil (approx. 3.6-fold) and Phenobarbital (approx. 4-fold). <u>Liver weights and weight of bile fluid excreted</u> (See Tables A6.10.3-1 and A6.10.3-2) A single dose of Fipronil or Phenobarbital had no statistically significant effect on liver weight or the weight of bile fluid excreted. However, 14 daily doses of 10 mg/kg bw/d Fipronil or of 80 mg/kg bw/d Phenobarbital clearly increased the weight of excreted bile during the 5-hour collection period. Liver weights (relative to terminal bodyweight) were increased by 22% and 24% over mean control group values after 14-day treatment with 10 mg/kg bw/d Fipronil and with 80 mg/kg bw/d Phenobarbital, respectively.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Rat biliary excretion of radiolabelled thyroxine**	
	Biliary excretion (see Tables A6.10.3-3, A6.10.3-4 and A6.10.3-5). After a single oral dosing, excretion of ^{125}I-T_4 (total radioactivity) via the bile within 5-hours post dosing was significantly higher ($p< 0.05$) in the Phenobarbital group only, although slight increases were also seen in the groups administered Fipronil at a single dose of 1 or 10 mg/kg bw. Similar results were obtained by group-wise comparison of the calculated biliary clearance values, although the increased value for the Phenobarbital group (2-fold increase over controls) was not statistically significant. Biliary clearance and the amount of bile excreted were significantly increased after treatment with 14 daily doses of 10 mg/kg bw/d Fipronil or of 80 mg/kg bw/d Phenobarbital. Although increased biliary clearance was also seen at the lower dose of Fipronil, statistical significance was not achieved. Characterisation of radiolabel in bile samples (see Table A6.10.3-6) HPLC analysis of the bile samples showed that most of the radioactivity recovered in untreated bile (80-90%) was neither free ^{125}I nor unconjugated ^{125}I-T_4. However, treatment of the samples with β-glucuronidase revealed that about 50% of the radioactivity was conjugated ^{125}I-T_4, either as the glucurono or sulfate conjugate. About 30% of the material, with a retention time of 6-9 minutes, was unidentified and may have represented other products of thyroxine metabolism. Furthermore, it was found in the 14-day dosing experiment that the biliary excretion of conjugated ^{125}I-T_4 during 0–5 hours was increased ca. 4.6-fold after Phenobarbital treatment, ca. 3-fold at the lower dose of Fipronil (1 mg/kg bw/d) and ca. 4-fold at the higher dose of Fipronil (10 mg/kg bw/d). These increases over the control levels after multiple doses were greater than that of the corresponding increases after the single doses. **Discussion:** This study showed that repeated oral administration for fourteen days of both 1 and 10 mg/kg of Fipronil and of 80 mg/kg bw/d of Phenobarbital increased biliary clearance of T_4 (thyroxine) in rats resulting in an increase in T_4-conjugated products. Responses with Fipronil were more marked after the higher dose level. Increased biliary clearance of T_4 has been shown to decrease T_4 blood levels which stimulates the pituitary to secrete and release more TSH. This, in turn, stimulates the thyroid follicular cells to produce more T_4 and exhibit hypertrophic and hyperplastic microscopic changes. A possible mechanism involved is hepatic accumulation of T_4 and induction of the hepatic conjugating enzyme uridine diphosphate glucuronyl transferase (UDP-GT) resulting in increased biliary excretion of T_4-glucuronide conjugate. The observed increase in T_4-conjugated products in the bile in this study supports the view that Fipronil could act via this mechanism.	
5.3 Conclusion	Fourteen daily oral doses of both 1 and 10 mg/kg bw/d of Fipronil to rats enhanced biliary clearance of T_4 (Thyroxine) resulting in an increase in T_4-conjugated products in the bile. The effect was more pronounced at the higher dose level and were higher than after 14 daily intraperitoneal doses of 80 mg/kg bw/d of Phenobarbital, the positive control.	

Section A6.10 **Annex Point IIIA, VI.7**	**Mechanistic studies** **- Rat biliary excretion of radiolabelled thyroxine**

5.3.1 Reliability	1
5.3.2 Deficiencies	None

Table A6.10.3-1 Group mean liver weight (% of terminal bodyweight)

Number of daily doses	Dose level (mg/kg bw/d)			
	Fipronil			Phenobarbital
	0	1	10	80
One	3.9 (100%)	4.2 (108%)	3.8 (97%)	3.7 (95%)
Fourteen	3.7 (100%)	3.9 (105%)	4.5* (122%)	4.6 (124%)

% of control in parenthesis; Statistical evaluation: * p<0.05

Table A6.10.3-2 Weight of bile fluid excreted during 0-5 hours post-dosing (g)

Number of daily doses	Dose level (mg/kg bw/d)			
	Fipronil			Phenobarbital
	0	1	10	80
One	2.91 (100%)	3.29 (113%)	3.31 (114%)	3.72 (128%)
Fourteen	4.28 (100%)	4.23 (99%)	6.02** (141%)	6.06*** (142%)

% of control in parenthesis; Statistical evaluation: ** p<0.01; *** p<0.001

Table A6.10.3-3 Proportions of radiolabel in liver, bile and whole blood (% of dose administered)

Number of daily doses	Tissue	Dose level (mg/kg bw/d)			
		Fipronil			Phenobarbital
		0	1	10	80
One	Liver	8.42 (100%)	9.33 (111%)	9.38 (111%)	8.07 (96%)
	Bile	6.48 (100%)	7.55 (117%)	8.61 (133%)	9.34* (144%)
	Whole blood	17.45 (100%)	19.28 (111%)	17.67 (101%)	12.95** (74%)
	% total	32.35 (100%)	36.14* (112%)	35.65 (110%)	30.37 (94%)
Fourteen	Liver	4.58 (100%)	8.23*** (180%)	7.50** (164%)	12.75*** (278%)
	Bile	3.78 (100%)	10.98*** (291%)	13.58*** (359%)	14.88*** (394%)
	Whole blood	8.48 (100%)	12.14* (143%)	8.40 (99%)	12.47** (147%)
	% total	16.84 (100%)	31.34*** (186%)	29.48*** (175%)	40.10*** (238%)

% of control in parenthesis; Statistical evaluation: * p<0.05; ** p<0.01; *** p<0.001

Table A6.10.3-4 Amount of ^{125}I-T_4 excreted in bile during 0-5 hours post-dose (total ng equivalents)

Number of daily doses	Dose level (mg/kg bw/d)			
	Fipronil			Phenobarbital
	0	1	10	80
One	14.1306 (100%)	16.8799 (120%)	18.4011 (130%)	21.2047 (150%)
Fourteen	8.1990 (100%)	23.1201 ~~(182%)~~ (282%)	28.0671 ~~(242%)~~ (342%)	33.1797 ~~(305%)~~ (405%)

% of control in parenthesis

X

Table A6.10.3-5 Biliary clearance of radioactivity after a single intravenous dose of ^{125}I-T_4 (ml/hour)

Number of daily doses	Dose level (mg/kg bw/d)			
	Fipronil			Phenobarbital
	0	1	10	80
One	0.799 (100%)	0.991 (124%)	1.027 (129%)	1.619 (203%)
Fourteen	2.421 (100%)	3.669 (152%)	9.830 (406%***)	4.356 (180%)

% of control in parenthesis; Statistical evaluation: *** p<0.001

Table A6.10.3-6 HPLC analysis: amount of conjugated ^{125}I-T_4 in pooled bile samples (results expressed as ng ^{125}I-T_4)

Number of daily doses	Dose level (mg/kg bw/d)			
	Fipronil			Phenobarbital
	0	1	10	80
One	20.1588 (100)	29.8059 (148)	31.5063 (156)	35.0131 (174)
Fourteen	11.5983 (100)	36.0189 (311)	46.7362 (403)	51.7349 (446)

% of control in parenthesis;
each value represents the total amount of radioactivity in the 12 individual bile samples, which were pooled to form one sample for HPLC analysis

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Date	April 2007
Materials and methods	Agree with applicant's version. **Revisions/amendments:** 1.1 Reference: *The Effect of ~~singe~~* single *and repeated oral doses of M&B 46030* (1 mg/kg/day and 10 mg/kg/day) 3.1 Test material: ~~*As given in section*~~ Fipronil M&B 46030 3.1.2 Specification: ~~*As given in section 2*~~ The substance was used as delivered by the sponsor 3.1.2.1 Description: ~~*White powder*~~ Creamy-yellowish crystalline powder 3.1.2.2 Purity: ~~*96.7%*~~ not mentioned in the study document. 3.1.2.3 Stability: *Stable* The stability was the responsibility of the sponsor 3.2.2 Strain: *Sprague-Dawley [Crl: CD(SD)]* (Crl: CD/BR)
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.11 **Other routes of administration**
No Annex Point

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	There are no other routes of exposure associated with this compound	
Undertaking of intended data submission [ ]		

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	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.12 **Annex Point IIA, VI.6.9**	**Medical data in anonymous form**

Section A6.12.1 **Annex Point IIA, VI.6.9.1**	**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.	**Official use only**

Section A6.12.2 **Annex Point IIA, VI.6.9.2**	**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.	**Official use only**

Section A6.12.3 **Annex Point IIA, VI.6.9.3**	**Health records** Company health records do not show any fipronil-related health effects.	**Official use only**

Section A6.12.4 **Annex Point IIA, VI.6.9.4**	**Epidemiological studies on the general population** Not available.	**Official use only**

Section A6.12.5 **Annex Point IIA, VI.6.9.5**	**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, XXXX, seems to be HPLC/MS.	**Official use only**

Section A6.12.6 **Annex Point IIA, VI.6.9.6**	**Sensitisation/allergenicity observations** No human cases of sensitisation / allergenicity deriving from fipronil have been reported to us.	**Official use only**

Section A6.12.7 **Annex Point IIA, VI.6.9.7**	**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. <u>Specific anti-convulsive therapy</u> Recommendations are based on anti-convulsive therapy as routinely administered to humans.	**Official use only**

	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.	

Section A6.12.8 **Annex Point IIA, VI.6.9.8**	**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.	**Official use only**

	EVALUATION BY COMPETENT AUTHORITIES
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Date	April 2007
Materials and methods	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.13 **Annex Point IIIA, VI.2**	**Toxic effects on livestock and pets**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	The use patterns of the biocidal products and their label instructions prevent any exposure to livestock or pets.	
Undertaking of intended data submission [ ]		

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

Section A6.14 **Other tests related to the exposure of humans** **Annex Point IIIA, III-X1.2**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	Data only required if available and if the use patterns of biocidal products make further studies necessary. Neither is the case with fipronil.	
Undertaking of intended data submission [ ]		

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

Section A6.15	**Food and feedingstuffs**
Annex Point IIIA, VI..4	

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [ ]**	
Limited exposure [x]	**Other justification [ ]**	
Detailed justification:	Cockroach products are recommended for use in such areas but only by professional operators. The products are gels placed as small droplets in cracks and crevices and the label states that it should be used in such a way that food or water could not become contaminated. Therefore there should be no significant contamination.	
Undertaking of intended data submission [ ]		

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2007
Materials and methods	Agree with applicant's version.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	acceptable
Remarks	The product may be applied in occupied premises, except during cooking activities. It is recommended to apply GOLIATH GEL in cracks and crevices, or in concealed locations inaccessible to man or domestic animals: behind refrigerators cupboards and shelves, under kitchen appliances (the application on hoods has not been evaluated), in electrical control boxes, voids and ducting and under bathroom fixtures etc. Spots should not be applied in areas where it will become submersed or likely to be removed by routine cleaning.
	COMMENTS FROM …
Date	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.16 **Annex Point IIIA, VI.3.5, XI.2**	**Any other test related to the exposure of the active substance to humans, in its proposed biocidal products, that are considered necessary may be required**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [x]**	
Detailed justification:	No further studies with Fipronil are considered to be required, since the toxicological profile of Fipronil is clearly identified with studies addressing sections 6.1 to 6.10 to establish risk assessment to humans for the proposed biocidal uses as an insecticide. There are no other biocidal product uses that would require additional studies.	
Undertaking of intended data submission [ ]		

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

Section A6.17 **Annex Point IIIA, VI.6**	**Tests to assess toxic effects of metabolites from treated plants**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	**Official use only**
Other existing data [ ]	**Technically not feasible [ ] Scientifically unjustified [x]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In chapter 3 of the TNsG on additional data requirements it is stated under point A6.17 that the submission tests to assess toxic effects to metabolites from treated plants may be required, if the active substance is to be used in products for action against plants. Since Fipronil is not be used in any biocide product intended for action against plants, therefore no tests are required.	
Undertaking of intended data submission [ ]		

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

Section A6.18 **Annex Point IIA, VI.6.10**	**Summary of mammalian toxicology and conclusions** **(RMS: This section A6.18 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.)**

Toxicokinetics and metabolism:

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 XXXX, XXX and XXXX 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, XXXX, XXXX and XXX 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 (XXXX), XXXX 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 XXXX. A proposed metabolic pathway in rats is presented below.

Figure 6.18/1 Proposed metabolic2profile of fipronil in the rat

XXXX

3

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 A6.18/1 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)	**Reference**
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	Cheng, T. (1995) [see III A6.2/04]
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	Ward, R.J. (1997a) [see III A6.2/05]
300 EC (300 g/l)**	0.82	0.15	7.0	1.07	1.03	0.215	5	Ward, R.J. (1997b) [see III A6.2/06]
50 SC (50 g/l)**	15	0.15	7.09	0.95	2.99	0.052	58	Ward, R.J. (1997c) [see III A6.2/07]
200 SC (200 g/l)**	1.19	0.05	No data	No data	9.92	0.42	24	Walters, K.A. and Brain, K.R. (1991) [see III A6.2/08]

* 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, resp.). 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. Fipronil was not a skin contact sensitizer in either the Magnusson and Kligman Maximisation or 3-fold induction Buehler tests.

Table A6.18/2 Summary of acute toxicity studies

Study, species	Fipronil purity	Sex	Result	Reference
Acute oral toxicity, rat	93%	Males Females	LD_{50}: 92 mg/kg bw LD_{50}: 103 mg/kg bw	XXXX. (1988) [see A6.1.1/01] KS
Acute dermal toxicity, rat	93%	Males and females	$LD_{50:}$ >2000 mg/kg bw	XXXX. (1988) [see A6.1.2/01]
Acute dermal toxicity, rabbit	96.7%	Males Females	LD_{50}: 445 mg/kg bw LD_{50}: 354 mg/kg bw	XXXX. (1992) [see A6.1.2/02] KS
Acute inhalation toxicity, rat (Fipronil technical)	95.4%	Males and females	LC_{50}: 0.68 mg/l	XXXX. (1991) [see A6.1.3/01]
Acute inhalation toxicity, rat (Fipronil technical, air-milled)	96.7%	Males Females	LC_{50}: 0.36 mg/l LC_{50}: 0.42 mg/l	XXXX. (1995) [see A6.1.3/02] KS
Skin irritation, rabbit	96.7%	Males and females	Not irritant	XXXX. (1993a) [see A6.1.4/01] KS
Eye irritation, rabbit	96.7%	Males and females	Not irritant	XXXX. (1993b) [see A6.1.4/02] KS
Skin Sensitisation (M&K test), Guinea pig	95.4%	Males and females	Not a sensitizer	XXXX. (1993); [see A6.1.5/01] KS

KS: Key Study

Short-term 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 clonic 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.

Table A6.18/3 Summary of short-term toxicity studies

Species	Duration and route	Fipronil purity	Dose levels	NO(A)EL	Reference
Rat	28-day oral diet	93%	0, 25, 50, 100, 200 and 400 ppm	<25 ppm (males: 3.4 mg/kg bw/d; females: 3.5 mg/kg bw/d)	XXXX (1990) [see A6.3.1/01]
Rabbit	21-day dermal	96.7%	0, 0.5, 1, 5 and 10 mg/kg bw/d	5 mg/kg bw/d	XXXX. (1993) [see A6.3.1/02]
Rat	90-day oral diet	95.4%	0, 1, 5, 30 and 300 ppm	5 ppm (males: 0.33 mg/kg bw/d; females: 0.37 mg/kg bw/d)	Holmes, P. (1991a) [see A6.4.1/01]
Dog	90-day oral capsule	95.4%	0, 0.5, 2 and 10 mg/kg bw/d	0.5 mg/kg bw/d	Holmes, P. (1991b); [see A6.4.1/02]
Dog	1-year oral capsule	96.8%	0, 0.2, 2 and 5 mg/kg bw/d	0.2 mg/kg bw/d	Holmes, P. (1992) [see A6.4.1/03]
Dog	1-year oral diet	95.7%	0, 0.075, 0.3, 1 and 3/2 mg/kg bw/d	0.3 mg/kg bw/d	Holmes, P. (1993) [see A6.4.1/04]

Genotoxicity

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 A6.18/5]. 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.

Table A6.18/4 Summary of genotoxicity studies – in vitro studies

Study type	Test system	Dose levels	Purity (%)	Result	Reference
Bacterial reverse mutation (Ames) test	*Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA100	0, 0.8, 4, 20, 100 and 500 µg/plate (test 1); 0, 25, 50, 100, 200 and 400 µg/plate (test 2) with and without S9-mix	95-97	Negative (toxicity observed at ≥ 400 µg/plate)	XXXX. (1988) [see A6.6.1/01]
	E.coli strain WP2 uvrA	0, 20, 100, 500, 2500, 5000 µg/plate	98.9	Negative (toxicity observed at ≥2500 µg/plate	XXXX. (2005) [see A6.6.1/02]
Chromosome aberrations in mammalian cells	Human lymphocytes	0, 75, 150 and 300 µg/ml with and without S9 mix	95-97	Negative (300 µg/ml was limit of solubility)	XXXX. (1988) [see A6.6.2/01]
	Chinese hamster lung	Without S9 mix 0, 30, 45 and 60 µg/ml (6-hour exposure); 0, 7.5, 15, 22.5 and 30 µg/ml (24-and/or 48-hour exposures) With S9 mix 0, 15, 30 and 60 µg/ml (6-hour exposure)	98.3	Positive at 6-hour exposure with and without S9 mix at toxic dose levels (60 µg/ml)	XXXX. (1995) [see A6.6.2/02]
Gene mutation in mammalian cells	Chinese hamster lung V79 cells	0, 0.8, 4, 20, 100 and 500 µg/ml with and without S9 mix	97.2	Negative (slight toxicity observed in second test at 100 and 500 µg/ml)	XXXX. (1993) [see A6.6.3/01]

Table A6.18/5 Summary of genotoxicity studies – in vivo studies

Study type	Test system	Dose levels (mg/kg bw)	Purity (%)	Result	Reference
Mouse micronucleus	CD-1 mice erythrocyte bone marrow cells	0, 1, 5 and 25	97.2	Negative (no toxicity to bone marrow cells)	XXXX. (1993) [see A6.6.4/01]
	CD-1 mice erythrocyte bone marrow cells	0, 12.5, 25 and 50	96.2	Negative (no toxicity to bone marrow cells)	XXXX. (1995) [see A6.6.4/02]
Unscheduled DNA synthesis	Rat primary hepatocytes	0, 12.5, 25, and 50	91.7	Negative	XXXX. (2004) [see A6.6.5/01] XXXX. (2005) [see A6.6.5/02]

Long term toxicity and 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.

Table A6.18/6 Summary of long term toxicity and oncogenicity

Study	Dose levels (ppm)	NO(A)EL	Reference
Rat combined chronic toxicity and oncogenicity	0, 0.5, 1.5, 30 and 300 ppm	NOAEL: 0.5 ppm (Males: 0.019 mg/kg bw/d; Females: 0.025 mg/kg bw/d)	XXXX. (1993) [see A6.5/01]
Mouse oncogenicity	0, 0.1, 0.5, 10, 30 and 60 ppm	NOEL: 0.5 ppm (Males: 0.055 mg/kg bw/d; Females: 0.063 mg/kg bw/d)	XXXX. (1992) [see A6.7/01]

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.

Table A6.18/7 Thyroid hormone function studies

Test	Species	Findings	Reference
Investigation of thyroxine clearance	Rat	Thyroxine clearance stimulated by 14 daily doses of 10 mg/kg bw/d of Fipronil. Differences in pharmacokinetic profile of ^{125}I-T_4 in treated animals versus controls (48% decrease in terminal half-life, 161% increase in clearance and 37% increase in the volume of distribution).	XXXX (1991a) [see A6.10/01]
Perchlorate discharge	Rat	Stimulation of thyroid follicular activity shown by slight increase in thyroid weight and increased accumulation of ^{125}I in thyroid. No evidence of inhibition of iodide efflux by potassium perchlorate.	XXXX (1991b) [see A6.10/02]
Effects of one and 14 oral doses of Fipronil on biliary excretion of ^{125}I-thyroxine in bile cannulated rats	Rat	Increased biliary clearance of T_4 and in T_4-conjugated biliary products after 14 daily doses of Fipronil in comparison to controls.	XXXX (1993) [see A6.10/03]

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).

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Reproduction and developmental toxicity studies

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).

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.

Table A6.18/8 Summary of reproduction and developmental toxicity

Study type Fipronil dose levels	Purity (%)	NOAEL/NOEL		Reference (DocID)
		Parental toxicity	Developmental / reproduction toxicity	
Rat 2-gen. reproduction tox. 0–3–30–300 ppm	95.4	NOAEL: 3 ppm Males: 0.25 mg/kg bw/d Females: 0.27 mg/kg bw/d	NOEL: 30 ppm 2.54 mg/kg bw/d 2.74 mg/kg bw/d	XXXX (1992) [see A6.8.2/01] XXXX. (1993) [see A6.8.2/02]
Rat prenatal tox. 0–1–4–20 mg/kg bw/d	95.1	NOAEL: 4 mg/kg bw/d	NOEL: 20 mg/kg bw/d	XXXX (1991) [see A6.8.1/01]
Rabbit prenatal tox. 0–0.1–0.2–0.5–1 mg/kg bw/d	95.4	NOAEL: 0.2 mg/kg bw/d	NOEL: 1.0 mg/kg bw/d	XXXX [see A6.8.1/02]

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 bwof 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.

90-day dietary neurotoxicity in rats

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).

Developmental neurotoxicity in rats

Groups of mated female Sprague Dawley rats treated with 0, 0.5, 10 or 200 ppm of Fipronil from Day 6 post coitum to Day 10 post partum inclusive. The study evaluated the effects of this continuous dietary treatment on their offspring. At the highest dose level of 200 ppm (corresponding to 15.63 mg/kg bw/day), mortalities (one on Day 6 and the other on Day 9 post partum) occurred together with an initial loss of bodyweight and reduction in food consumption. At this clearly maternally toxic dose, there was a statistically significant increase in the number of pups found dead at birth and reduced pup and litter survival by Day 4 post partum. Furthermore, pup weight gain to weaning was reduced and was associated with a slight delay in development of tooth eruption, vaginal patency and preputial separation were seen. Other effects included a decrease in the maximum response voltage required to elicit an auditory startle response and a delay in swimming development. No neuropathological changes were seen. At the 10 ppm intermediate dose level, there was no maternal toxicity. The only finding originally considered to be treatment related in the offspring was a slight reduction in bodyweights during lactation. Body weight gain was not affected during this period. This finding is considered to be unlikely to be related to treatment given the clear lack of effects on pup weight at the higher dose level of 30 ppm in the rat multigeneration reproduction study. No evidence of any developmental neurotoxicity was seen. Therefore, the NOAEL for both developmental neurotoxicity and general toxicity was 10 ppm (corresponding to 0.91 mg/kg bw/day).

14-day repeat dose neurotoxicity in dogs

In the dog repeat dose oral neurotoxicity study, a capsular dose of 20 mg/kg of Fipronil was given to female Beagle dogs for 14 consecutive days and followed by a 28-day off-dose recovery period. Treatment caused marked initial inappetance resulting in marked weight loss by Day 3 post-dosing. Clear evidence of functional neurotoxicity (convulsion, head nodding, tremors, stiffening of the limbs, gait abnormalities and depressed or exaggerated reflexes and placing/postural responses) occurred between 5 and 13 days post dosing. Neurological examination revealed a range of abnormalities in addition to those noted in the veterinary examinations, although marked variation in the type, frequency, and timing of response was apparent. Considering repeated dosing at 20 mg/kg bw/day is most likely approaching a lethal dose and the severity of the toxicity observed during treatment, including a significant body weight loss and an almost complete lack of food consumption, only minimal evidence of any residual effects were observed during the recovery period. In most cases, complete recovery was noted 12 days or less after cessation of treatment. At the end of the 28-day recovery period, only one animal, which had received treatment for 13 days, displayed a slightly exaggerated flexor response. However, an effect on flexor response was not seen in the subchronic or chronic toxicity studies in dogs. Therefore, the relevance of the slightly exaggerated flexor response observation is of questionable significance. Histopathological examination of the nervous tissues following the 28-day reversibility period revealed no evidence of changes for any animal in the study.

Conclusion on neurotoxicity

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, short-term, chronic or perinatal exposures to Fipronil. In general, both rats and dogs 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.

Table A6.18/9 Summary of neurotoxicity studies

Study type Dose levels	Purity (% w/v)	No Observed Effect Level (NOEL)		Reference
		General toxicity	Neurotoxicity	
Rat oral acute neurotoxicity 0, 0.5, 5.0 and 50 mg/kg bw	96.7	0.5 mg/kg bw	0.5 mg/kg bw	XXXX (1993) [see A6.9/01]
Rat oral acute neurotoxicity 0, 2.5, 7.5 and 25 mg/kg bw	97.9	2.5 mg/kg bw	2.5 mg/kg bw	XXXX. (1997) [see A6.9/02]
Rat 90-day neurotoxicity 0, 0.5, 5.0 and 150 ppm	96.7	NOAEL: 5 ppm M: 0.3 mg/kg bw/d F: 0.4 mg/kg bw/d	150 ppm M: 8.9 mg/kg bw/d F: 10.8 mg/kg bw/d	XXXX. (1993) [see A6.9/03]
Rat developmental neurotoxicity 0, 0.5, 10 and 200 ppm	96.1	10 ppm (0.9 mg/kg bw/day)	10 ppm 0.9 mg/kg bw/day	XXXX. (1995) XXXX [not a key study]
Dog 14-day oral neurotoxicity 0 and 20 mg/kg bw/d	95.4	Not applicable	Not applicable	XXXX. (1991) XXXX [not a key study]

TOXICOLOGICAL SUMMARY

Low doses of Fipronil are rapidly and extensively absorbed from the gastrointestinal tract following oral exposure. Fipronil is widely distributed in the body with a preference for fatty tissues and is extensively metabolised. Elimination of Fipronil (mainly via biliary excretion of Fipronil metabolites) is slow, which seems to be partly due to retention of Fipronil residues in tissues and also due to its significant enterohepatic circulation. Fipronil is toxic by inhalation, in contact with skin and if swallowed. It is not a skin or eye irritant and not a skin sensitiser. In toxicity studies with fipronil, clinical signs of toxicity were observed that are consistent with its interaction at the GABA-gated chloride channel in the nervous system. These clinical signs are noted in all species tested and were found to be reversible in neurobehavioral testing in rats and dogs. No histopathological findings are observed in the nervous system after acute, short term, chronic or perinatal exposures to fipronil. Other evidence of fipronil toxicity included changes in the liver of mice and rats, and the induction of follicular cell tumours in the rat thyroid at high dose levels. The absence of a genotoxicity/mutagenicity potential of Fipronil was demonstrated in a battery of in-vitro and in-vivo tests. Mechanistic studies indicated that the thyroid tumours occur via a rat-specific, non-genotoxic (threshold) mechanism involving the disturbance of the hypothalamic-pituitary-thyroid axis. The sensitivity of the rat to the induction of thyroid tumours compared to the relative insensitivity noted in other species is well known and indicates that these lesions have no practical relevance for human risk assessment. No evidence developmental toxicity or of impaired fertility was found in reproduction toxicity studies with Fipronil.

	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	